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(54) Title: USE OF GPR54 LIGANDS FOR TREATMENT OF REPRODUCTIVE DISORDERS, PROLIFERATIVE DISORDERS, AND FOR CONTRACEPTION

(57) Abstract: The present invention features methods of treating a reproductive disorder such as central precocious puberty, polycystic ovarian disease, endometriosis, irregular periods, or uterine fibroids, a proliferative disorder involving a sex steroid dependent cancer such as a prostate cancer, breast cancer, uterine cancer, or testicular cancer, or ovarian cancer in a patient by administering an agonist of GPR54 or a compound that continuously interferes with a biological activity of a GPR54 polypeptide and decreases this biological activity or by administering a compound that continuously occupies a GPR54 polypeptide and decreases a biological activity of the GPR54 polypeptide. Such compounds may also be used in methods of contraception and in the preparation for *in vitro* fertilization.

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PATENT
ATTORNEY DOCKET NO. 00786/481WO2

USE OF GPR54 LIGANDS FOR TREATMENT OF REPRODUCTIVE DISORDERS, PROLIFERATIVE DISORDERS, AND FOR CONTRACEPTION

Background of the Invention

In general, the invention relates to the medical management of reproductive disorders and contraception.

The pulsatile secretion of gonadotropin hormone releasing hormone (GnRH) from the hypothalamus generates the normal reproductive axis of boys and girls during the neonatal period (Waldhauser et al., Eur. J. Pediatr. 137:71-74, 1981), and again at puberty (Boyar et al., J. Clin. Invest. 54:609-618, 1974), whereas a marked decrease in the amplitude of its secretion causes the quiescence of reproductive activity characteristic of childhood (Ross et al., J. Clin. Endocrinol. Metab. 57:288-293, 1983; Wu et al., J. Clin. Endocrinol. Metab. 70:629-637, 1990; and Dunkel et al., J. Clin. Endocrinol. Metab. 74:890-897, 1992).

Abnormalities in secretion of GnRH, or impairment of an individual's response to GnRH, can lead to a variety of disorders including idiopathic hypogonadotropic hypogonadism ("IHH"), microphallus, i.e., a stretched penile length that is less than 2.5 cm at birth, and hypospadius, i.e., the incomplete fusion of the penile urethra. In addition, abnormalities in the secretion of GnRH or in a person's response to GnRH can result in hypogonadotropic hypogonadism in men and women, hypothalamic amenorrhea, e.g., the absence of the normal initiation of menses or the cessation of menses for at least 3 months in a woman with previously normal menstrual cycles, delayed puberty, and possibly polycystic ovarian disease. In addition, it is possible to induce hypogonadotropic hypogonadism with GnRH analogues for therapeutic purposes in the settings of central precocious puberty, prostate cancer, endometriosis, and uterine fibroids, as well as suppressing the gonad for purposes of contraception and treatment of polycystic ovarian disease.

Furthermore, GnRH and/or its analogues which produce hypogonadotropic hypogonadism also may be used in preparation for *in vitro* fertilization (IVF). Fifteen

percent of all couples have difficulty with conception. In general, IVF is a means to achieve pregnancy using the following steps: (1) superovulation of the female partner, (2) retrieval of the mature follicles, (3) joining of the eggs and sperm for conception *in vitro*, (4) culturing and support of the embryos, and (5) transfer of embryos to the uterus. Superovulation is often achieved by first administering a GnRH agonist for two weeks to inhibit endogenous gonadotropin production (i.e. induce hypogonadotropic hypogonadism) prior to induction of ovulation, thereby maximizing control of the menstrual cycle. Alternatively, GnRH antagonists can result in more rapid desensitization and hence production of a hypogonadotropic state than GnRH agonists, and may reduce the risk of ovarian hyperstimulation syndrome (Paulson and Marrs, Curr. Probl. Obstet. Gynecol. Infert. 10:497, 1986; and Inany and Aboulghar, Cochrane Database Syst. Rev. 4:CD001750, 2001).

IHH is a condition in which puberty fails to occur in the face of low/normal gonadotropins and the absence of any anatomic or functional cause. As nearly all patients with IHH respond to exogenous GnRH, the defect in this disorder is likely at the level of the secretion or action of the hypothalamic peptide GnRH. Typically, IHH becomes apparent in late adolescence with failure of sexual development heralded by the absence of the normal appearance of secondary sex characteristics. The condition is characterized by (1) complete or partial absence of endogenous GnRH-induced LH pulsations (Boyar et al., J. Clin. Endocrinol. Metab. 43:1268-1275, 1976; Crowley et al., Rec. Prog. Horm. Res. 41:473-531, 1985; Santoro et al., Endocr. Rev. 7:11-23, 1986; Spratt et al., J. Clin. Endocrinol. Metab. 64:283-291, 1987; and Pitteloud et al., J. Clin. Endocrinol. Metab. 87:152-160, 2002), (2) a lack of any anatomic cause on radiographic imaging of the hypothalamus and pituitary (Whitcomb and Crowley, "Male Hypogonadotropic Hypogonadism." In: Veldhuis, editor, Endocrinology and Metabolism Clinics of North America, Philadelphia: WB Saunders Co. 125-143, 1993; and Ross et al., J. Clin. Endocrinol. Metab. 57:288-293, 1983), (3) normal baseline and reserve testing of the remaining hypothalamic-pituitary axes, and (4) a generally normal response to physiologic replacement with exogenous GnRH, localizing the defect to an abnormality of GnRH synthesis, secretion, or action

(Santoro et al., Endocr. Rev. 7:11-23, 1986; Crowley and McArthur, J. Clin. Endocrinol. Metab. 51:173-175, 1980; and Hoffman and Crowley, N. Engl. J. Med. 307:1237-1241, 1982).

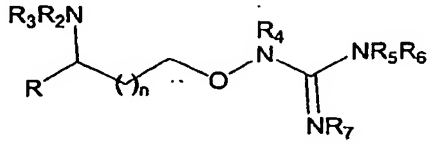
Given the number of disorders that are associated with abnormal GnRH levels or action, a continuing need exists for new and improved compounds that can be used to treat such disorders.

Summary of the Invention

The present invention features methods of treating a reproductive disorder such as central precocious puberty, polycystic ovarian disease, endometriosis, irregular periods, or uterine fibroids or a proliferative disorder involving a sex steroid dependent cancer such as a prostate cancer, breast cancer, uterine cancer, or testicular cancer or ovarian cancer in a patient by administering a compound alters biological activity of a GPR54 polypeptide or that continuously interferes with a biological activity of a GPR54 polypeptide and decreases this biological activity or by administering a compound that continuously occupies a GPR54 polypeptide and that affects a biological activity of the GPR54 polypeptide. Such compounds may also be used in methods of contraception and in the preparation for *in vitro* fertilization.

Accordingly, the first aspect of the invention features a method for treating a reproductive disorder. This method involves administering to a patient in need thereof an effective amount of a compound that alters a GPR54 biological activity, where the compound is selected from

Formula I



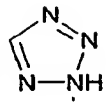
where

R is CO_2R_1 or Z;

R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;

R_2 and R_3 is C(=O)Me;

n = 0 – 2; and

Z = 

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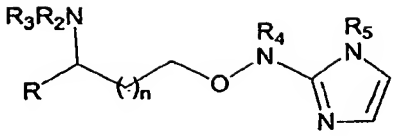
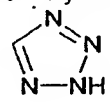
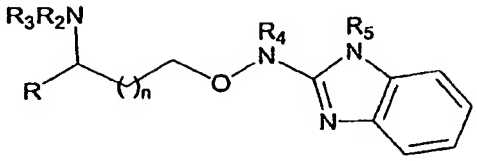


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

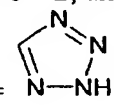
Z = 

or

Formula III

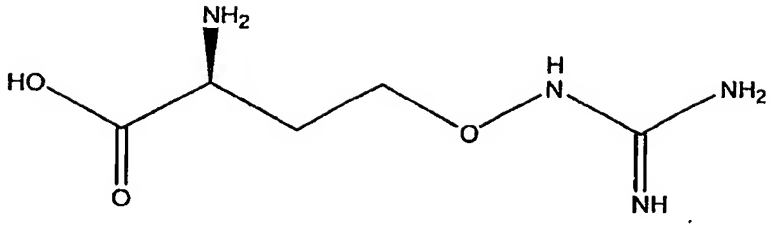


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

Z = 

and where the administering alters the GPR54 biological activity.

In a desirable embodiment of the first aspect of the invention, the administering decreases the GPR54 biological activity. In another desirable embodiment of the first aspect of the invention, the compound is:



The second aspect of the invention features another method for treating a reproductive disorder. This method involves administering to a patient in need thereof an effective amount of a compound that continuously interferes with a GPR54 biological activity, where the administering decreases the GPR54 biological activity.

The third aspect of the invention features a further method for treating a reproductive disorder. This method involves administering to a patient in need thereof an effective amount of a compound that specifically binds to a GPR54 polypeptide, where the compound continuously occupies the GPR54 polypeptide, and where the administering decreases a biological activity of the GPR54 polypeptide.

The fourth aspect of the invention features yet another method for treating a reproductive disorder. This method involves administering to a patient in need thereof an effective amount of a compound that specifically binds to a GPR54 polypeptide, where the administration results in a luteinizing hormone level in the patient that is below a basal luteinizing hormone level observed in the patient prior to the administration.

In a desirable embodiment of the second, third, and fourth aspects of the invention, the compound is a peptide, a peptide derivative, or a small molecular weight agonist or antagonist of the GPR54 polypeptide.

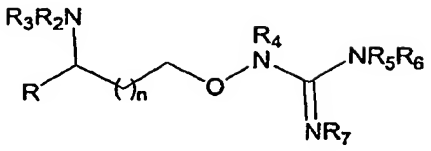
In a desirable embodiment of the second aspect of the invention, continuous interference with the GPR54 biological activity includes continuous administration of an effective amount of the compound to the patient.

In a desirable embodiment of the third aspect of the invention, continuously occupying the GPR54 polypeptide involves continuous administration of an effective amount of the compound to the patient.

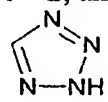
In other desirable embodiments of the second, third, and fourth aspects of the

invention, the compound is selected from

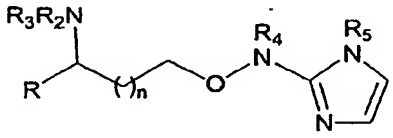
Formula I



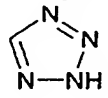
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



Formula II

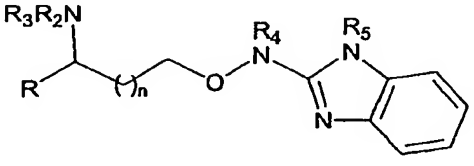


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



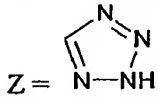
or

Formula III

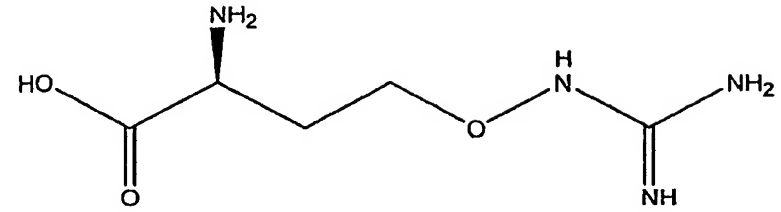


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;

n = 0 – 2; and



Desirably, the compound is:

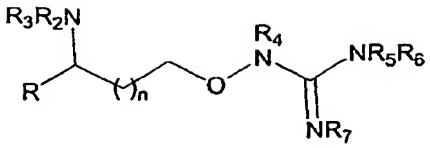


In additional desirable embodiments of the second, third, and fourth aspects of the invention, the compound is a kisspeptin polypeptide or a derivative thereof. A kisspeptin polypeptide desirably includes amino acids 68-121, 94-121, 107-121, 108-121, 109-121, 112-121, 113-121, or 114-121 of SEQ ID NO:1. In a more desirable embodiment, the kisspeptin polypeptide consists of amino acids 112-121 of SEQ ID NO:1.

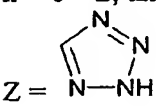
In desirable embodiments of any one of the first four aspects of the invention, the reproductive disorder is central precocious puberty, polycystic ovarian disease, endometriosis, irregular periods, or uterine fibroids. In other desirable embodiments of the first four aspects of the invention, the administering reduces the level of GnRH, a gonadotropin, or a sex steroid in the patient. Desirably, the administering induces a state of hypogonadotropic hypogonadism in the patient. In further desirable embodiments of any one of the first four aspects of the invention, the administering involves intramuscular, intravenous, subcutaneous, transdermal, transvaginal, or oral administration. Desirably, transvaginal administration involves a vaginal ring.

The fifth aspect of the invention features a method for treating a proliferative disorder. This method involves administering to a patient in need thereof an effective amount of a compound that alters a GPR54 biological activity, where the compound is selected from

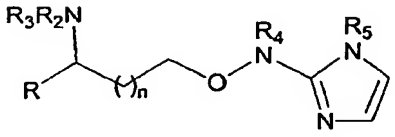
Formula I



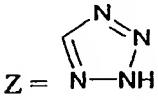
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



Formula II

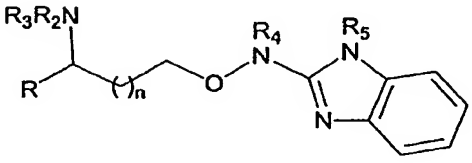


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

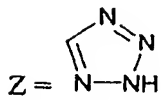


or

Formula III

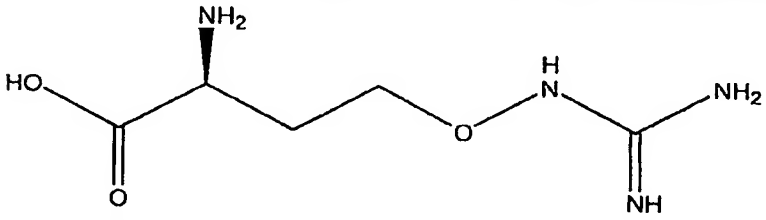


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



and where the administering alters the GPR54 biological activity.

In a desirable embodiment of the fifth aspect of the invention, the administering decreases the GPR54 biological activity. In another desirable embodiment of the fifth aspect of the invention, the compound is:



The sixth aspect of the invention features another method for treating a proliferative disorder. This method involves administering to a patient in need thereof an effective amount of a compound that continuously interferes with a GPR54 biological activity, where the administering decreases the GPR54 biological activity.

The seventh aspect of the invention features a further method for treating a proliferative disorder. This method involves administering to a patient in need thereof an effective amount of a compound that specifically binds to a GPR54 polypeptide, where the compound continuously occupies the GPR54 polypeptide, and where the administering decreases a biological activity of the GPR54 polypeptide.

The eighth aspect of the invention features yet another method for treating a proliferative disorder. This method involves administering to a patient in need thereof an effective amount of a compound that specifically binds to a GPR54 polypeptide, where the administration results in a luteinizing hormone level in the patient that is below a basal luteinizing hormone level observed in the patient prior to the administration

In desirable embodiments of any one of the fifth, sixth, seventh, or eighth aspects of the invention, the proliferative disorder is prostate cancer, breast cancer, uterine cancer, ovarian cancer, or testicular cancer.

In desirable embodiments of the sixth, seventh, or eighth aspects of the

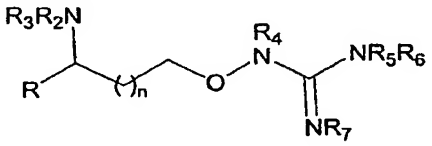
invention, the compound is a peptide, a peptide derivative, or a small molecular weight agonist or antagonist of the GPR54 polypeptide.

In a desirable embodiment of the sixth aspect of the invention, continuous interference with the GPR54 biological activity involves continuous administration of an effective amount of the compound to the patient.

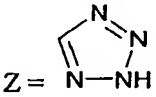
In a desirable embodiment of the seventh aspect of the invention, continuously occupying the GPR54 polypeptide involves continuous administration of an effective amount of the compound to the patient.

In other desirable embodiments of the sixth, seventh, and eighth aspects of the invention, the compound is selected from

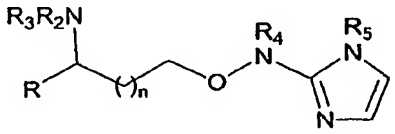
Formula I



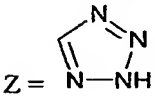
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



Formula II

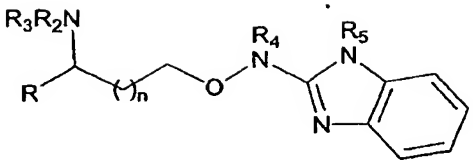


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

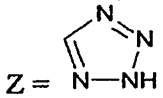


or

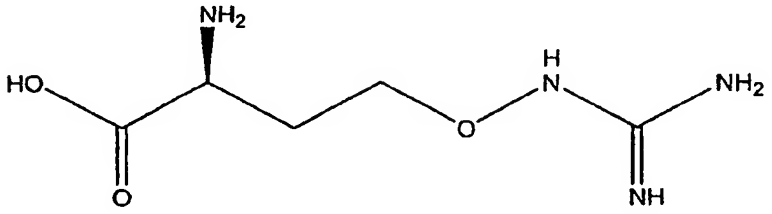
Formula III



where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



Desirably, the compound is:

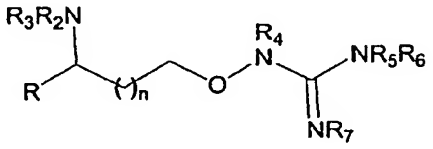


In other desirable embodiments of the sixth, seventh, and eighth aspects of the invention, the compound is a kisspeptin polypeptide or a derivative thereof. A kisspeptin polypeptide desirably includes amino acids 68-121, 94-121, 107-121, 108-121, 109-121, 112-121, 113-121, or 114-121 of SEQ ID NO:1. In a more desirable embodiment, the kisspeptin polypeptide consists of amino acids 112-121 of SEQ ID NO:1.

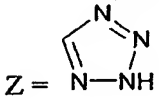
In desirable embodiments of the fifth, sixth, seventh, and eighth aspects of the invention, the administering reduces the level of GnRH, a gonadotropin, or a sex steroid in the patient. In other desirable embodiments of the fifth, sixth, seventh, and eighth aspects of the invention, the administering involves intramuscular, intravenous, subcutaneous, transdermal, transvaginal, or oral administration. Desirably, transvaginal administration involves a vaginal ring.

The ninth aspect of the invention features a method for contraception in a patient. This method involves administering to a patient in need thereof an effective amount of a compound that alters a GPR54 biological activity, where the compound is selected from

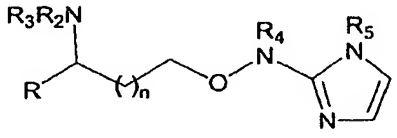
Formula I



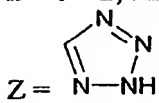
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



Formula II

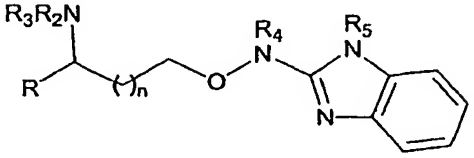


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

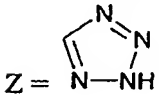


or

Formula III

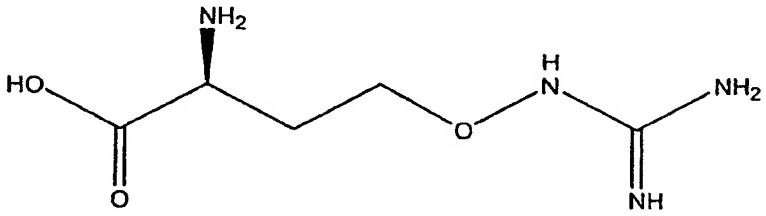


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
$n = 0 - 2$; and



and where the administering alters the GPR54 biological activity.

In a desirable embodiment of the ninth aspect of the invention, the administering decreases the GPR54 biological activity. In another desirable embodiment of the ninth aspect of the invention, the compound is:



The tenth aspect of the invention features another method for contraception in a patient. This method involves administering to a patient in need thereof an effective amount of a compound that continuously interferes with a GPR54 biological activity, where the administering decreases the GPR54 biological activity.

The eleventh aspect of the invention features a further method for contraception in a patient. This method involves administering to a patient in need thereof an effective amount of a compound that specifically binds to a GPR54 polypeptide, where the compound continuously occupies the GPR54 polypeptide, and where the administering decreases a biological activity of the GPR54 polypeptide.

The twelfth aspect of the invention features yet another method for contraception in a patient. This method involves administering to a patient in need thereof an effective amount of a compound that specifically binds to a GPR54 polypeptide, where the administration results in a luteinizing hormone level in the patient that is below a basal luteinizing hormone level observed in the patient prior to the administration.

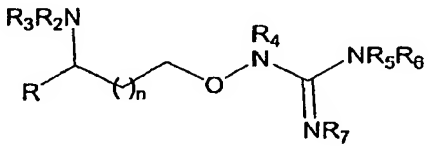
In desirable embodiments of the tenth, eleventh, and twelfth aspects of the invention, the compound is a peptide, a peptide derivative, or a small molecular weight agonist or antagonist of the GPR54 polypeptide.

In a desirable embodiment of the tenth aspect of the invention, continuous interference with the GPR54 biological activity involves continuous administration of an effective amount of the compound to the patient.

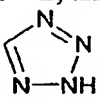
In a desirable embodiment of the eleventh aspect of the invention, continuously occupying the GPR54 polypeptide involves continuous administration of an effective amount of the compound to the patient.

In other desirable embodiments of the tenth, eleventh, and twelfth aspects of the invention, the compound is selected from

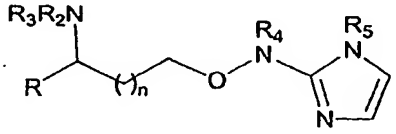
Formula I



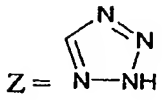
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

Z = 

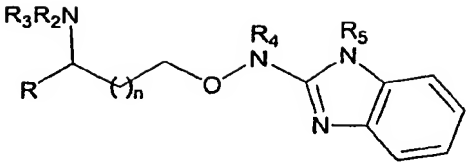
Formula II



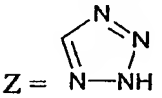
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



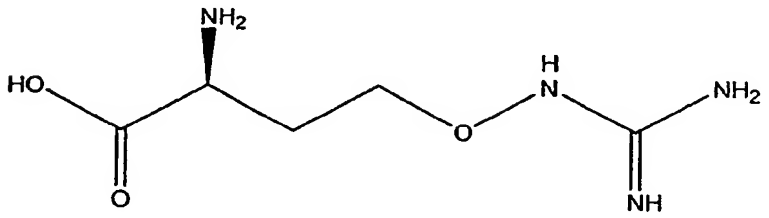
or
Formula III



where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



Desirably, the compound is:



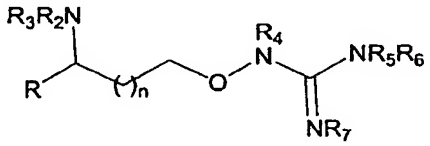
In further desirable embodiments of the tenth, eleventh, and twelfth aspects of the invention, the compound is a kisspeptin polypeptide or a derivative thereof. A kisspeptin polypeptide desirably includes amino acids 68-121, 94-121, 107-121, 108-121, 109-121, 112-121, 113-121, or 114-121 of SEQ ID NO:1. In a more desirable embodiment, the kisspeptin polypeptide consists of amino acids 112-121 of SEQ ID NO:1.

In desirable embodiments of the ninth, tenth, eleventh, and twelfth aspects of the invention, the administering reduces the level of GnRH, a gonadotropin, or sex steroid in the patient. In other desirable embodiments of the ninth, tenth, eleventh,

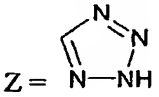
and twelfth aspects of the invention, the administering includes intramuscular, intravenous, subcutaneous, transdermal, transvaginal, or oral administration. Desirably, transvaginal administration involves a vaginal ring.

In the thirteenth aspect, the invention features a method for treating infertility in a patient. This method involves administering to a patient in need thereof an effective amount of a compound that alters a GPR54 biological activity, where the compound is selected from

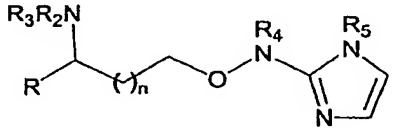
Formula I



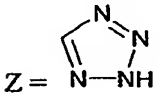
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



Formula II

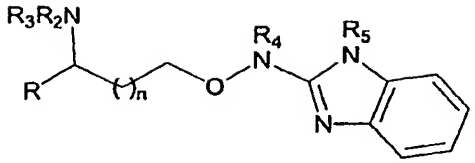


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

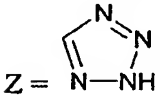


or

Formula III

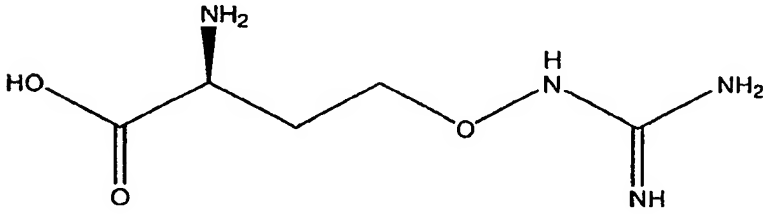


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



and where the administering alters the GPR54 biological activity.

In a desirable embodiment of the thirteenth aspect of the invention, the administering decreases the GPR54 biological activity. In another desirable embodiment of the thirteenth aspect of the invention, the compound is:



The fourteenth aspect of the invention features another method for treating infertility in a patient. This method involves administering to a patient in need thereof an effective amount of a compound that continuously interferes with a GPR54 biological activity, where the administering decreases the GPR54 biological activity.

The fifteenth aspect of the invention features an additional method for treating infertility in a patient. This method involves administering to a patient in need thereof an effective amount of a compound that specifically binds to a GPR54 polypeptide, where the compound continuously occupies the GPR54 polypeptide, and where the administering decreases a biological activity of the GPR54 polypeptide.

The sixteenth aspect of the invention features a further method for treating infertility in a patient. This method involves administering to a patient in need thereof

an effective amount of a compound that specifically binds to a GPR54 polypeptide, where the administration results in a luteinizing hormone level in the patient that is below a basal luteinizing hormone level observed in the patient prior to the administration

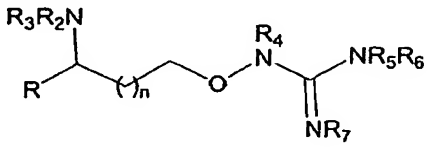
In desirable embodiments of the fourteenth, fifteenth, and sixteenth aspects of the invention, the compound is a peptide, a peptide derivative, or a small molecular weight agonist or antagonist of the GPR54 polypeptide.

In a desirable embodiment of the fourteenth aspect of the invention, continuous interference with the GPR54 biological activity involves continuous administration of an effective amount of the compound to the patient.

In a desirable embodiment of the fifteenth aspect of the invention, continuously occupying the GPR54 polypeptide involves continuous administration of an effective amount of the compound to the patient.

In other desirable embodiments of the fourteenth, fifteenth, and sixteenth aspects of the invention, the compound is selected from

Formula I



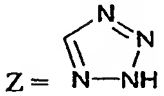
where

R is CO_2R_1 or Z;

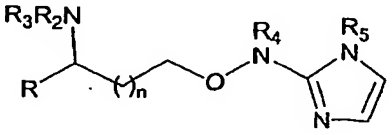
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;

R_2 and R_3 is C(=O)Me;

n = 0 – 2; and

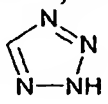
Z = 

Formula II



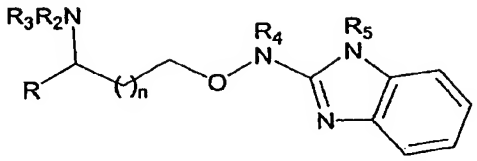
where

R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

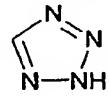
Z = 

or

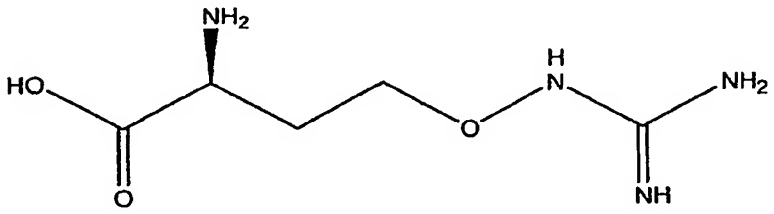
Formula III



where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

Z = 

Desirably, the compound is:



In other desirable embodiments of the fourteenth, fifteenth, and sixteenth aspects of the invention, the compound is a kisspeptin polypeptide or a derivative thereof. A kisspeptin polypeptide desirably includes amino acids 68-121, 94-121, 107-121, 108-121, 112-121, or 114-121 of SEQ ID NO:1. In a more desirable embodiment, the kisspeptin polypeptide consists of amino acids 112-121 of SEQ ID NO:1.

In desirable embodiments of the thirteenth, fourteenth, fifteenth, and sixteenth

aspects of the invention the administering reduces the level of GnRH, a gonadotropin, or a sex steroid in the patient. In other desirable embodiments of the thirteenth, fourteenth, fifteenth, and sixteenth aspects of the invention, the administering includes intramuscular, intravenous, subcutaneous, transdermal, transvaginal, or oral administration. Desirably, transvaginal administration involves a vaginal ring.

In addition desirable embodiments of any one of the first sixteen aspects of the invention, the patient is a human.

In additional aspects, the invention features kisspeptin and GPR54 knockout mice. In further aspects, the invention features pharmaceutical compositions containing a therapeutically effective dose of the peptides and small molecules disclosed herein, as well as kits containing such pharmaceutical compositions and instructions for administration.

Definitions

By a "kisspeptin polypeptide," as used herein, is meant a polypeptide that is substantially identical to, or is identical to, a mammalian *KiSS-1* gene product or a fragment thereof. Desirable kisspeptin polypeptides are C-terminal fragments of a mammalian *KiSS-1* gene product or a derivative of such an amino acid sequence. In other desirable embodiments, the kisspeptin polypeptide is a human kisspeptin polypeptide, for example, one containing the amino acid sequence MNSLVSWQLLLFLCATHFGEPLEKVASVGNSRPTGQQLESLGLLAPGEQSLPC TERKPAATARLSRRGTSLSPPPESSGSRQQPGLSAPHSRQIPAPQGAVLVQREK DLPNYNWNSFGLRFGKREAAPGNHGRSAGRGWGAGAGQ (SEQ ID NO:1) or amino acids 68-121, 94-121, 107-121, 108-121, 109-121, 112-121, 113-121, or 114-121 of the amino acid sequence of SEQ ID NO:1 (or of GenBank Accession No. AAM78592, CAH7122.6, or NP_002247). In further desirable embodiments, a kisspeptin polypeptide may include amino acids 68-119 of the murine *KiSS-1* (GenBank Accession No. AF472576) gene product, or a fragment thereof. In addition, the kisspeptin polypeptide desirably can alter a GPR54 biological activity.

As used herein, by a "GPR54 polypeptide" or a "GPR54 protein" is meant an

amino acid sequence that is substantially identical or is identical to the polypeptide encoded by GenBank Accession Number AY029541, AF343725, NM_032551, or AY253981, or a fragment thereof. Desirably, a GPR54 polypeptide or GPR54 protein has a GPR54 biological activity.

As used herein, by a "GPR54 nucleic acid" is meant a nucleic acid molecule that encodes a polypeptide that is substantially identical or is identical to the polypeptide encoded by GenBank Accession Number AY029541, AF343725, NM_032551, or AY253981, or a fragment thereof. Desirably, this nucleic acid sequence encodes a polypeptide that has a GPR54 biological activity.

By a "GPR54 biological activity," as used herein, is meant a GPR54-dependent alteration in luteinizing hormone (LH) levels, intracellular calcium release, a change in the phosphorylation state of a polypeptide in a signaling pathway regulated by GPR54, a GPR54-dependent alteration in arachidonic acid release from a cell, a GPR54-dependent alteration in phosphatidylinositol turnover, or an alteration in endogenous GnRH secretion. A GPR54 biological activity may be regulated by a compound, e.g., a peptide or a small molecule, that binds to GPR54 or that alters the expression of a GPR54 nucleic acid molecule or polypeptide. Further, compounds that alter the activity or expression of components of a GPR54 signaling pathway may also alter a GPR54 biological activity. Guidance for assaying protein interactions, function, and expression may be found in, for example, Ausubel et al. (*Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001).

By "altering a GPR54 biological activity," as used herein, is meant a decrease or an increase in a GPR54 biological activity, relative to a control. Preferably, the decrease in GPR54 biological activity is at least 20%, 40%, 50%, or 75% below that of an untreated control as measured by any standard assay known in the art, including the assays described herein. More desirably, the biological activity of a GPR54 polypeptide is decreased by 80%, 90%, 95%, or even 100% below that of an untreated control. Alternatively, an alteration in GPR54 biological activity may be an increase in GPR54 biological activity that is at least 20%, 40%, 50%, or 75% above that of an untreated control as measured by any standard assay known in the art, including the

assays described herein. More desirably, the increase in GPR54 biological activity is at least 80%, 90%, 95%, or even 100% above that of an untreated control. Such responses can be monitored using, for example, calcium release assays, determining the phosphorylation state of a polypeptide in a signaling pathway regulated by GPR54, arachidonic acid release assays, phosphatidylinositol turnover assays, an IP-One assay, and an alteration in endogenous GnRH secretion.

By "decreasing a GPR54 biological activity," as used herein, is meant a decrease in a GPR54 biological activity, relative to a control. Preferably, the decrease in GPR54 biological activity is at least 20%, 40%, 50%, or 75% below that of an untreated control as measured by any standard assay known in the art, including the assays described herein. More desirably, the biological activity of a GPR54 polypeptide is decreased by 80%, 90%, 95%, or even 100% below that of an untreated control. Such responses can be monitored using, for example, luteinizing hormone (LH) levels, calcium release assays, determining the phosphorylation state of a polypeptide in a signaling pathway regulated by GPR54, arachidonic acid release assays, phosphatidylinositol turnover assays, an IP-One assay, and an alteration in endogenous GnRH secretion.

By "continuous interference" or "continuously interfering," as used herein in reference to a compound, is meant a compound that binds or otherwise reduces the level or activity of a GPR54 polypeptide or a component of a GPR54 signaling pathway and that thereby decreases the luteinizing hormone (LH) level in a subject below the basal LH level observed in the subject prior to administration of the compound. Desirably, a continuously interfering compound binds or reduces the activity of a component of a GPR54 signaling pathway in the same cell that expresses the GPR54 polypeptide or the continuously interfering compound affects the expression or activity of a ligand of GPR54. In other desirable embodiments, the compound is administered continuously to a subject for at least four hours or intermittently over a period of at least four hours to decrease the LH level in the subject below the basal level observed prior to administration of the compound. In another desirable embodiment the decrease in LH level below the basal level is at

least 3%, 5%, 8%, 10%, 12%, 15%, 20%, 25%, 30%, 50%, 70%, 80%, 90%, 95%, or even 100% below the basal level. Further, the decrease in LH level below the basal level desirably is observed after the compound is administered continuously or intermittently for at least 5, 10, 12, 24, 36, 48, 60, 72, 84, or 96 hours. LH levels can be determined using standard techniques as described herein. In other desirable embodiments, continuous interference results in a decrease in GnRH secretion or activity.

By "continuous occupancy" or "continuously occupying," as used herein in reference to a compound, is meant a compound that binds a GPR54 polypeptide and that decreases the luteinizing hormone (LH) level in a subject below the basal LH level observed in the subject prior to administration of the compound. Desirably, the compound is administered continuously to a subject for at least four hours or intermittently over a period of at least four hours to decrease the LH level in the subject below the basal level observed prior to administration of the compound. In another desirable embodiment the decrease in LH level below the basal level is at least 3%, 5%, 8%, 10%, 12%, 15%, 20%, 25%, 30%, 50%, 70%, 80%, 90%, 95%, or even 100% below the basal level. Further, the decrease in LH level below the basal level desirably is observed after the compound is administered continuously or intermittently for at least 5, 10, 12, 24, 36, 48, 60, 72, 84, or 96 hours. LH levels can be determined using standard techniques as described herein. In other desirable embodiments, continuous occupancy results in a decrease in GnRH secretion or activity.

"Continuous administration" as used herein, refers to uninterrupted administration of a compound for at least four hours. Desirably the compound is continuously administered for at least 10, 12, 24, 36, 48, 60, 72, 84, or 96 hours. In other desirable embodiments, continuous administration spans 1, 2, or 3 weeks, 1, 2, 3, 4, 5, 6, 9, or 12 months, or even 2, 3, or more years. However, the compound may also be continuously administered until the subject is free of the disease being treated or until contraception or preparation for *in vitro* fertilization is no longer indicated. Continuous administration may involve uninterrupted intravenous, intramuscular, or

subcutaneous infusion of a pharmaceutical composition containing the compound, as well as transdermal or transvaginal infusion of the compound. Desirably, transvaginal infusion involves the use of a vaginal ring that gradually releases the compound over time. Further, continuous administration may involve oral administration of a compound, e.g., in a time-release formulation.

"Intermittent administration" as used herein refers to non-continuous administration of a compound. Desirably the compound is intermittently administered once every 1, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 72, or 96 or more hours. The intermittent administration interval desirably is repeated for 10, 12, 24, 36, 48, 60, 72, 84, or 96 or more hours. In other desirable embodiments, the intermittent administration interval is repeated for 1, 2, or 3 weeks, 1, 2, 3, 4, 5, 6, 9, or 12 months, or even 2, 3, or more years. However, the compound may also be intermittently administered until the subject is free of the disease being treated or until contraception or preparation for *in vitro* fertilization is no longer indicated. Intermittent administration may involve intravenous, intramuscular, or subcutaneous infusion of a pharmaceutical composition containing the compound, as well as transdermal or transvaginal infusion of the compound. Further, intermittent administration may involve oral administration of a compound.

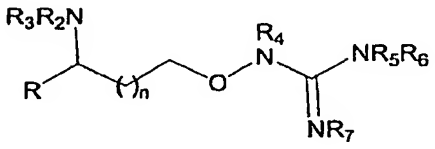
By an "increased endogenous GnRH secretion" as used herein, is meant an increase in a GnRH secretion from a cell, relative to a control. Preferably, the increase in GnRH secretion that is at least 20%, 40%, 50%, or 75% above that of an untreated control as measured by any standard assay known in the art, including the assays described herein. More desirably, the increase in GnRH secretion is at least 80%, 90%, 95%, or even 100% above that of an untreated control. Such responses can be monitored, for example, by exposing a cell expressing a GPR54 polypeptide to a test sample and assaying the biological activity of the GPR54 polypeptide relative to a control not exposed to the test sample, or by exposing a cell expressing a GnRH-regulated reporter gene, e.g., luciferase, to a test sample and assaying reporter gene expression relative to a control cell not exposed to the test sample.

By a "candidate compound" or "test compound" is meant a chemical, be it

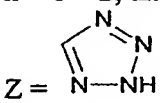
naturally-occurring or artificially-derived, that is surveyed for its ability to alter a biological activity of GPR54, e.g., in one of the assay methods described herein. Candidate or test compounds include, for example, peptides, polypeptides, synthetic organic molecules, naturally-occurring organic molecules, nucleic acid molecules, and components thereof. Exemplary candidate compounds may be members of the RFamide (neuropeptides terminating in -Arg-Phe-NH_2) and RWamide families (Clements et al., Biochem. Biophys. Res. Commun. 284:1189-1198 (2001).

A "small molecular weight agonist of GPR54" as used herein desirably is a compound encompassed by one of the following formulas.

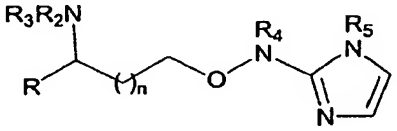
Formula I



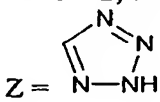
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



Formula II

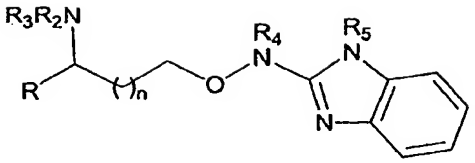


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

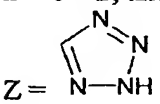


and

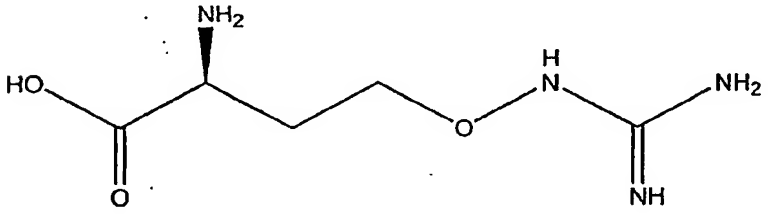
Formula III



where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



An exemplary small molecular weight agonist of GPR54 is



The term "lower alkyl" as used herein means alkyl groups of from 1 to about 7 carbon atoms that consist of a straight, branched or cyclic configuration. Desirable examples of lower alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, s- and t-butyl, pentyl, hexyl, heptyl, cyclopropyl, cyclobutylmethyl, cycloheptyl, and the like.

The term "aryl" as used herein, is phenyl, 1-naphthyl, or 2-naphthyl.

The term "heteroaryl" as used herein means 5- and 6-membered heterocyclic aromatic systems containing about 1-3 heteroatoms taken from the group including O, N or S.

By "substantially identical" is meant a polypeptide or nucleic acid exhibiting at least 50%, preferably 60%, 70%, 75%, or 80%, more preferably 85%, 90% or 95%, and most preferably 99% identity to a reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 10 amino acids, desirably at least 15 contiguous amino acids, more desirably at least 20,

25, 50, 75, 90, 100, 150, 200, 250, 300, or 350 contiguous amino acids, and most desirably the full-length amino acid sequence. For nucleic acids, the length of comparison sequences will generally be at least 45 contiguous nucleotides, desirably at least 60 contiguous nucleotides, more desirably at least 75, 150, 225, 275, 300, 450, 600, 750, 900, or 1000 contiguous nucleotides, and most desirably the full-length nucleotide sequence.

Sequence identity may be measured using sequence analysis software on the default setting (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software may match similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine, valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

Multiple sequences may also be aligned using the Clustal W(1.4) program (produced by Julie D. Thompson and Toby Gibson of the European Molecular Biology Laboratory, Germany and Desmond Higgins of European Bioinformatics Institute, Cambridge, UK) by setting the pairwise alignment mode to "slow," the pairwise alignment parameters to include an open gap penalty of 10.0 and an extend gap penalty of 0.1, as well as setting the similarity matrix to "blosum." In addition, the multiple alignment parameters may include an open gap penalty of 10.0, an extend gap penalty of 0.1, as well as setting the similarity matrix to "blosum," the delay divergent to 40%, and the gap distance to 8.

Substantially identical nucleic acid sequences also include nucleic acid sequences that hybridize to the complement of a given nucleic acid sequence under high stringency hybridization conditions. Exemplary high stringency hybridization conditions include hybridization at approximately 42°C in about 50% formamide, 0.1 mg/ml sheared salmon sperm DNA, 1% SDS (Sodium Dodecyl Sulfate), 2X SSC (Sodium Citrate Buffer), 10% Dextran Sulfate, a first wash at approximately 65°C in about 2X SSC, 1% SDS, followed by a second wash at approximately 65°C in about

0.1X SSC. Alternatively, high stringency hybridization conditions may include hybridization at approximately 42°C in about 50% formamide, 0.1 mg/ml sheared salmon sperm DNA, 0.5% SDS, 5X SSPE, 1X Denhardt's, followed by two washes at room temperature in 2X SSC, 0.1% SDS, and two washes at between 55-60°C in 0.2X SSC, 0.1% SDS.

By "specifically binds" as used herein in reference to a compound is meant an increased affinity of a compound for a GPR54 polypeptide relative to an equal amount of any other polypeptide present in a sample or a patient. The compound desirably has an affinity for a GPR54 polypeptide that is least 2-fold, 5-fold, 10-fold, 30-fold, or 100-fold greater than for an equal amount of any other polypeptide present in a sample or a patient.

By a "functional fragment," as used herein, is meant an amino acid sequence that is substantially identical to a fragment, e.g., 5, 10, 15, 20, 15, 30, 50, 75, or 100 contiguous amino acids, of a polypeptide encoded by GenBank Accession No. AY029541, AF343725, NM_032551, or AY253981. In more desirable embodiments, a "functional fragment" is identical to 5, 10, 15, 20, 15, 30, 50, 75, or 100 contiguous amino acids of GenBank Accession No. AY029541, AF343725, NM_032551, or AY253981, or may be the entire amino acid sequence of the polypeptide encoded by GenBank Accession No. AY029541, AF343725, NM_032551, or AY253981. In addition, a "functional fragment" of a polypeptide has at least one biological activity of the full-length GPR54 polypeptide.

The term "derivative" as used herein in connection with an amino acid sequence, refers to a peptide or polypeptide that, while containing at least one alteration, is substantially identical in structure and maintains at least one of the biological activities of the peptide or polypeptide on which it is based. Desirably, a derivative of a kisspeptin polypeptide maintains the ability to bind a GPR54 polypeptide. Exemplary alterations include N-terminal acetylation, glycosylation, biotinylation, and the addition of a polyethylene glycol molecule. A derivative of a kisspeptin polypeptide may also be a cyclic peptide or include an amino acid substitution, such as the substitution of one amino acid for another within the same

class (e.g., with a nonpolar, uncharged polar, charged polar, or phenyl R group), substitution of an L-amino acid for a D-amino acid, or substitution of a standard amino acid for a non-standard amino acid or amino acid analog. Non-standard amino acids and amino acid analogs include, α,α -disubstituted amino acids, N-alkyl amino acids, lactic acids, C-α-methyl amino acids, β-methyl amino acids, β-alanine, norvaline, norleucine, 4-aminobutyric acid, orithine, hydroxyproline, sarcosine, citrulline, cysteic acid, cyclohexylalanine, 2-aminoisobutyric acid, 6-aminohexanoic acid, t-butylglycine, phenylglycine, o-phosphoserine, N-acetyl serine, N-formylmethionine, 3-methylhistidine, adamantylalanine, 3-benzothienylalanine, 4,4'-biphenylalanine, 3,3-diphenylalanine, homophenylalanine, 2,6-dichlorobenzyltyrosine, cyclohexyltyrosine, 7-benzyloxytryptophan, tri-tert-butyltryptophan, homotryptophan, 3-(-Anthracenyl)-L-alanine, L-p-iso-propylphenylalanine, L-thyroxine, and 3,3',5-triiodo-L-thyronine.

By "purified" is meant separated from other components that naturally accompany it. Typically, a factor is substantially pure when it is at least 50%, by weight, free from proteins, antibodies, and naturally-occurring organic molecules with which it is naturally associated. Preferably, the factor is at least 75%, more preferably, at least 90%, and most preferably, at least 99%, by weight, pure. A substantially pure factor may be obtained by chemical synthesis, separation of the factor from natural sources, or production of the factor in a recombinant host cell that does not naturally produce the factor. Proteins, vesicles, and organelles may be purified by one skilled in the art using standard techniques, such as those described by Ausubel et al. (*Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001). The factor is preferably at least 2, 5, or 10 times as pure as the starting material, as measured using polyacrylamide gel electrophoresis, column chromatography, optical density, HPLC analysis, or Western analysis (Ausubel et al., *Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001). Preferred methods of purification include immunoprecipitation, column chromatography such as immunoaffinity chromatography, magnetic bead immunoaffinity purification, and panning with a plate-bound antibody.

"Proliferative disorder," as used herein, refers to any genetic change within a differentiated cell that results in the abnormal proliferation of a cell. Such changes include mutations in genes involved in the regulation of the cell cycle, of growth control, or of apoptosis and can further include tumor suppressor genes and proto-oncogenes. Desirably, a proliferative disorder is affected by sex steroid levels. As such, sex steroid dependent prostate, breast, uterine, ovarian, or testicular cancers are desirable examples of proliferative disorders.

"Reproductive disorder" as used herein, refers to a malfunction or malformation of the reproductive system. Exemplary reproductive disorders associated with increased endogenous GnRH secretion or activity include, without limitation, central precocious puberty, endometriosis, polycystic ovarian disease, and uterine fibroids.

"Central precocious puberty," as used herein, refers to the appearance of any primary or secondary indications of puberty in an individual at a time that is greater than two standard deviations before the onset of puberty in individuals of the same sex in a population.

"Polycystic ovary disease," as used herein, refers to a clinical syndrome characterized by chronic oligoamenorrhea and hyperandrogenism, either evident on physical examination (i.e. acne or hirsutism) or with elevated levels of serum androgens in the bloodstream. This disorder affects 5-7% of all reproductive age women, making it the most common endocrinopathy in this group. The ovaries are studded with atretic follicles. Close to 50% of patients with polycystic ovary disease ("PCOS") are found to have frank diabetes, impaired glucose tolerance, or impaired insulin resistance at the time of diagnosis. In addition to abnormalities in the insulin/glucose axis, the neuroendocrine axis also appears to be abnormal in PCOS. This disorder is further described in: Kazer et al., "Circulating luteinizing hormone pulse frequency in women with polycystic ovary syndrome," J. Clin. Endocrinol. Metab. 65:233-236, 1987; Waldstreicher et al., "Hyperfunction of the hypothalamic-pituitary axis in women with polycystic ovarian disease: indirect evidence for partial gonadotroph desensitization," J. Clin. Endocrinolo. Metab. 66:165-172, 1988; and

Dunaif, "Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis," Endocr. Rev. 18:774-800, 1997.

"Prostate cancer," as used herein, refers to a neoplasm of the prostate. This is the most common form of cancer in men, outside of non-melanoma skin cancer. Androgen deprivation via hormone therapy has traditionally been offered to men with locally advanced or metastic prostate cancer, although new combinations of therapies are currently being studied. Androgen ablation is accomplished via a GnRH agonist, which desensitizes the gonadotrophs, and ultimately results in loss of gonadotroph stimulation to the testicle. This disorder is further described in: Garnick, "Prostate cancer: screening, diagnosis, and management," Ann. Intern. Med. 118:804-818, 1993; Kramer et al., "Prostate cancer screening: what we know and what we need to know," Ann. Intern. Med. 119:914-923, 1993; and Wirth et al., "Bicalutamide (Casodex) 150 mg as immediate therapy in patients with localized or locally advanced prostate cancer significantly reduces the risk of disease progression," Urology 58:146-51, 2001.

"Endometriosis," as used herein, refers to the presence of endometrial glands and stroma outside the endometrial cavity and uterine musculature. Endometriosis is found in 21-48 % of women undergoing laparoscopy for infertility. The most common symptoms of endometriosis are pelvic pain, dysmenorrhea, dyspareunia, abnormal menstrual bleeding, and infertility. This disorder is further described in: Conn and Crowley, "Gonadotropin-releasing hormone and its analogs," Annu. Rev. Med. 45:391-405, 1994; Sangi-Haghpeykar and Poindexter, "3rd Epidemiology of endometriosis among parous women" Obstet. Gynecol. 85:983-92, 1995; Dlugi et al., "Depot (leuprolide acetate for depot suspension) in the treatment of endometriosis: a randomized, placebo-controlled, double-blind study. Lupron Study Group," Fertil. Steril. 54:419-27, 1990; and Ling, "Randomized controlled trial of depot leuprolide in patients with chronic pelvic pain and clinically suspected endometriosis. Pelvic Pain Study Group," Obstet. Gynecol. 93:51-58, 1999.

"Irregular periods," as used herein, refers to a menstrual bleeding frequency that is outside the normal range for a population. The normal menstrual bleeding

frequency is generally 25 to 35 days. Desirably, irregular periods occur at a frequency that is one standard deviation outside the normal menstrual bleeding frequency for a population.

"Uterine fibroids," as used herein, refer to benign leiomyomas or myomas arising from the smooth muscle cells of the uterus. Uterine fibroids are present in 25% of reproductive aged women. Genetics, hormones, and growth factors all play a role in the formation and growth of these tumors. The most common symptoms are excessive bleeding, pain, and infertility. This disorder is further described in: Buttram and Reiter, "Uterine leiomyomata: etiology, symptomatology, and management," Fertil. Steril. 36:433-434, 1981; Friedman et al., "A randomized, double-blind trial of a gonadotropin releasing-hormone agonist (leuprolide) with or without medroxyprogesterone acetate in the treatment of leiomyomata uteri," Fertil. Steril. 49:404-409, 1988; Carr et al., "An evaluation of the effect of gonadotropin-releasing hormone analogs and medroxyprogesterone acetate on uterine leiomyomata volume by magnetic resonance imaging: a prospective, randomized, double blind, placebo-controlled, crossover trial," J. Clin. Endocrinol. Metab. 76:1217-23, 1993; Conn and Crowley, "Gonadotropin-releasing hormone and its analogs," Annu. Rev. Med. 45:391-405, 1994; and Stewart, "Uterine fibroids," Lancet 357:293-298, 2001.

The present invention provides a number of advantages. For example, current treatments for central precocious puberty, prostate cancer, endometriosis, uterine fibroids, preparation for *in vitro* fertilization, and contraception generally use GnRH or GnRH analogs to produce a state of hypogonadotropic hypogonadism for therapeutic purposes. However, most currently available GnRH analogues are difficult to administer, especially orally. In contrast, the claimed methods encompass the use of small molecular weight regulators of GPR54 which may be administered orally as well as parentally to produce a similarly hypogonadotropic state and would thereby simplify the treatment of such reproductive conditions. Moreover, these methods encompass the use of novel contraceptive compounds.

Other features and advantages of the invention will be apparent from the following Detailed Description, the drawings, and the claims.

Brief Description of the Drawings

FIGURE 1A is a graph showing the effect of central administration of vehicle (n=4), 30 μg metastin (n=3), 100 μg metastin (n=4) or 100 μg metastin with acyline pretreatment (n=3) on GnRH release in juvenile orchidectomized rhesus monkeys, as reflected by plasma LH levels (mean±SEM).

FIGURE 1B is a graph showing the comparison of mean±SEM LH concentrations following central vehicle or metastin injections in juvenile orchidectomized rhesus monkeys (n=3-4). Bars with different letters differ ($P \leq 0.05$).

FIGURE 2 is a graph showing the effects of kisspeptin-10 and kisspeptin–54 (1 nmol, delivered ICV) on serum levels of LH in a mouse, measured at 30 min following a bolus injection (* $p < 0.001$ vs. vehicle alone).

FIGURE 3 is a graph showing the effects of different doses of kisspeptin-54 (metastin) (ranging from 0 to 5 nmol delivered ICV) on serum levels of LH in a mouse, measured 30 min following a bolus injection (* $p < 0.01$ vs. vehicle; ** $p < 0.001$ vs. vehicle; + $p < 0.001$ vs. 0.001 pmol metastin).

FIGURES 4A and 4B are a series of graphs showing the effects of metastin (50 pmol delivered ICV) or its vehicle alone in a mouse, coupled with pretreatment with either a GnRH antagonist, acyline (50 μg, sc) or its vehicle alone. Figure 4A depicts LH data and Figure 4B depicts FSH data (S/V = saline/vehicle, S/M = saline/metastin, A/V = acyline/vehicle, A/M = acyline/metastin; * $p < 0.001$ S/M vs. all other treatments).

FIGURES 5A-5F are a series of images showing the distribution of *KiSS-1* mRNA-expressing cells in the hypothalamus of a mouse. *KiSS-1* mRNA-expressing cells are indicated by clusters of white dots, corresponding to clusters of silver grain where the labeled RNA probe has been concentrated. *KiSS-1* mRNA-containing cells were observed in the anteroventral periventricular nucleus (Figure 5A), the periventricular nucleus (Figure 5B), anterodorsal preoptic nucleus (Figure 5C), the medial amygdala (Figure 5D), and the arcuate nucleus (Figures 5E and 5F) (3V, Third ventricle; AC, anterior commissure; OT, optic tract; OX, optic chiasm).

FIGURE 6 is a graph showing the effect of continuous administration of metastin 45-54 on LH release in agonadal juvenile male rhesus monkeys, in which pituitary responsiveness to GnRH had been previously heightened by pulsatile GnRH treatment. The dark circles represent LH levels (mean±SEM) from three monkeys receiving 100 μg/hour metastin 45-54 over a 98 hour infusion period (shaded horizontal bar). The open circles represent mean LH levels during vehicle infusion. Administration of metastin 45-54 or vehicle was initiated at 1000 hours (10 AM) on day 1. The white arrow indicates iv (intravenous) administration of the last GnRH priming pulse at 0800 hours (8 AM) on day 1. The black arrow indicates iv administration of a single bolus of metastin 45-54 at 0900 hours (9 AM) on day 1.

FIGURE 7 is a graph showing daytime (0900 hour (9 AM), open bars) and nighttime (2200 hours (10 PM), closed bars) plasma LH levels (mean±SEM) from three monkeys on day 2, 3, and 4 of a continuous infusion of 100 μg/hour metastin 45-54 (Met) or vehicle (Veh).

FIGURE 8 is a graph showing the effect of single sequential boluses of metastin 45-54 (Met, black arrow), NMDA (grey arrow), and GnRH (white arrow) on plasma LH concentrations (mean+SEM) during the last 3 hours of the 98 hour iv infusion of metastin 45-54 (shaded horizontal box) at a dose of 100 μg/hour compared with the LH response to the same bolus of metastin 1 hour before (day 1) and 21 hours after (day 6) the continuous metastin infusion. The asterisk ("*") indicates a value significantly different from the pre-injection mean. LH responses to metastin, NMDA and GnRH during infusion of vehicle were robust, but have been omitted for clarity (N=3).

FIGURE 9 is a graph showing the ano-genital distance (in cm) of Gpr54 knockout (Gpr54ko), Gpr54 heterozygous (Gpr54het), wild-type (wt), kisspeptin heterozygous (Kiss1het), and kisspeptin knockout (Kiss1ko) mice. (Minimum of eight mice per group.)

FIGURE 10 is a graph showing the time to vaginal opening (in days) in Gpr54 knockout (Gpr54ko), Gpr54 heterozygous (Gpr54het), wild-type (wt), kisspeptin heterozygous (Kiss1het), and kisspeptin knockout (Kiss1ko) mice. (Minimum of

eight mice per group.) Some kisspeptin knockout animals never achieved vaginal opening and are not represented in this figure.

FIGURES 11A and 11B are pictures showing the reproductive organs of mice. Ovaries and uteri are shown in FIGURE 11A and testes are shown in FIGURE 11B. The reproductive organs were obtained from Gpr54 knockout (Gpr54-/-), Gpr54 heterozygous (Gpr54+/-), wild-type (WT), kisspeptin heterozygous (Kiss1+/-), and kisspeptin knockout (Kiss1-/-) mice.

FIGURE 12 is a graph showing male gonad (testicular) weight (in mg) of Gpr54 knockout (Gpr54ko), Gpr54 heterozygous (Gpr54het), wild-type (wt), kisspeptin heterozygous (Kiss1het), and kisspeptin knockout (Kiss1ko) mice. (Minimum of eight mice per group.)

FIGURE 13 is a graph showing female gonad (ovarian) weight (in mg) of Gpr54 knockout (Gpr54ko), Gpr54 heterozygous (Gpr54het), wild-type (wt), kisspeptin heterozygous (Kiss1het), and kisspeptin knockout (Kiss1ko) mice. (Minimum of eight mice per group.)

FIGURES 14A and 14B are a series of graphs showing the baseline LH (FIGURE 14A) and FSH (FIGURE 14B) levels in male Gpr54 knockout (Gpr54-/-), Gpr54 heterozygous (Gpr54+/-), wild-type (wt), kisspeptin heterozygous (Kiss1+/-), and kisspeptin knockout (Kiss1-/-) mice. (Minimum of eight mice per group.)

FIGURE 15 is a graph showing male mouse LH levels before (dark bars) and thirty minutes after (light grey bars) injection with the kisspeptin C-terminal decapeptide YNWNSFGLRY-amide (SEQ ID NO:2). LH levels in male Gpr54 knockout (Gpr54-/-), Gpr54 heterozygous (Gpr54+/-), wild-type (wt), kisspeptin heterozygous (Kiss1+/-), and kisspeptin knockout (Kiss1-/-) mice are shown. (Minimum of eight mice per group.)

FIGURE 16A is a map of the vector used to stably express GPR54 in Chinese Hamster Ovary (CHO) cells.

FIGURE 16B is an image of a Northern blot showing high-level expression of GPR54 mRNA in cells transfected with the vector shown in FIGURE 16A. An empty vector control is also shown.

FIGURE 17 is a schematic diagram illustrating the IP-One assay described herein.

FIGURE 18 is a graph showing total intracellular inositol phosphates measured using myo-[2-3H]-inositol as the substrate.

FIGURE 19 is another graph showing that kisspeptin stimulates IP accumulation in a dose-dependent manner in CHO cells stably transfected with the vector shown in FIGURE 16A.

FIGURES 20A –20C are a series of graphs showing the statistics from high throughput screening with the CHO cells stably transfected with the vector shown in FIGURE 16A. Controls are shown in FIGURES 20B and 20C.

FIGURES 21A and 21B are graphs showing that LDN-21810 stimulates IP accumulation in a dose dependent manner. FIGURE 21A shows results obtained using the CHO cell line stably transfected with the vector shown in FIGURE 16A (and expressing GPR54) and FIGURE 21B shows results obtained from the empty vector control cell line.

FIGURE 22 is the structure of compound LDN-21810.

Detailed Description

Identifying the signals which modulate pulsatile GnRH secretion or a cell's response to GnRH can be used to understand and design therapeutic approaches (both stimulatory and suppressive) for the numerous human disorders that might benefit from either increasing or decreasing GnRH secretion, including idiopathic hypogonadotropic hypogonadism, amenorrhea, microphallus, microspadia, polycystic ovarian disease, endometriosis, uterine fibroids, and prostate cancer, as well as treatments for infertility and contraception (Conn and Crowley, "Gonadotropin-releasing hormone and its analogues," N. Engl. J. Med. 324:93-103, 1991).

The genetic and molecular basis for idiopathic hypogonadotropic hypogonadism (IHH) without anosmia, a condition characterized by the failure of the anterior pituitary to produce gonadotropins, was previously largely unknown save defects in the GnRH receptor. Most patients carrying the diagnosis of IHH respond to

exogenous administration of gonadotropin hormone releasing hormone (GnRH), a peptide normally produced by the hypothalamus. Therefore, the pathophysiologic defect of IHH has been assumed to be hypothalamic in origin. However, due to the infertility caused by untreated gonadotropin deficiency and the resultant small families, genetic approaches aimed at identifying the genes involved in IHH had been difficult to employ in this disease model.

Abnormalities in GnRH secretion or an individual's response to GnRH can lead to a variety of other disorders, including amenorrhea, microphallus, microspadia, and polycystic ovarian disease. In addition, induction of hypogonadotropic hypogonadism by suppression of endogenous GnRH secretion, is a valuable therapy for central precocious puberty, endometriosis, uterine fibroids, prostate cancer, contraception, and as a preparation for in vitro fertilization.

The identification of GPR54 as a key modulator of normal GnRH secretion not only enables its use in identifying and characterizing compounds that may be used to treat IHH, but also compounds that may be used to decrease GnRH secretion and produce hypogonadotropic hypogonadism that has already been proven useful as a treatment for several reproductive disorders such as central precocious puberty, prostate cancer, endometriosis, polycystic ovarian disease, uterine fibroids, as a potential contraceptive agent, and as a preparation for *in vitro* fertilization. Such compounds include the various kisspeptin polypepides described herein, such as metastin, as well as LDN-21810 and its derivates.

When administered as a single bolus to mice metastin elicits a robust LH response, mediated through GnRH release from the hypothalamus (Gottsch et al., "A Role for Kisspeptins in the Regulation of Gonadotropin Secretion in the Mouse," Endocrinology 145:4073-4077, 2004). These observations were also made in rats (Navarro et al., "Developmental and Hormonally Regulated Messenger Ribonucleic Acid Expression of KiSS-1 and its Putative Receptor, GPR54, In Rat Hypothalamus and Potent Luteinizing Hormone-Releasing Activity of KiSS-1 Peptide," Endocrinology 145:4565-4574, 2004; Matsui et al., "Peripheral Administration of Metastin Induces Marked Gonadotropin Release and Ovulation in the Rat," Biochem.

Biophys. Res. Comm. 320:383-388, 2004; Thompson et al., "Central and Peripheral Administration of Kisspeptin-10 Stimulates the Hypothalamic-Pituitary-Gonadal Axis," J. Neuroendocrinology 16:850-858, 2004; Irwig et al., "Kisspeptin Activation of Gonadotropin Releasing Hormone Neurons and Regulation Of KiSS-1 mRNA in the Male Rat," Neuroendocrinology 80:264-272, 2004; and Navarro et al., "Advanced Vaginal Opening and Precocious Activation of the Reproductive Axis by KiSS-1 Peptide, the Endogenous Ligand of GPR54," J. Physiology 561:379-386, 2004), and agonadal, juvenile, male monkeys.

When administered as an infusion to ovariectomized, E_2-treated sheep (4 hr) (Messager et al., "Kisspeptin Directly Stimulates Gonadotropin-Releasing Hormone Release via G Protein-Coupled Receptor 54," Proc. Natl. Acad. Sci. USA 102:1761-1766, 2005), and human males (90 min) (Dhillo et al., "Kisspeptin-54 Stimulates the Hypothalamic-Pituitary Gonadal Axis in Human Males," J. Clin. Endocrinol. Metab. doi:10.1210/jc.2005-1468), metastin also stimulates LH release. Outside of these single boluses and brief infusions, chronic, intermittent administration of metastin induces early sexual maturation in immature female rats (Navarro et al., "Advanced Vaginal Opening and Precocious Activation of the Reproductive Axis by KiSS-1 Peptide, the Endogenous Ligand of GPR54," J. Physiology 561:379-386, 2004) and sustained and precocious GnRH release in juvenile male monkeys. However, prior to the experiments described herein, the effect of long-term continuous administration of a kisspeptin polypeptide on GnRH secretion and activity was unknown.

The following examples are provided for the purpose of illustrating the invention and should not be construed as limiting.

Example 1

Characterization of GPR54 Ligands

GPR54 is a member of the rhodopsin family of G protein-coupled receptors with sequence homologies to members of the galanin receptor family, having 28, 30, and 30% amino acid identity with human GalR1, GalR2, and GalR3 respectively (Muir et al., "AXOR12, a novel human G protein-coupled receptor, activated by the

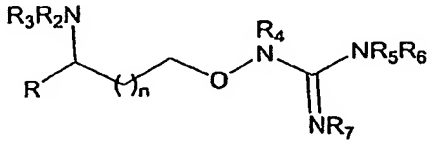
peptide Kiss-1," J. Biol. Chem. 276:28969-28975, 2001). Although galanin does not bind to GPR54, (Muir et al., "AXOR12, a novel human G protein-coupled receptor, activated by the peptide Kiss-1," J. Biol. Chem. 276:28969-28975, 2001; Lee et al., "Discovery of a receptor related to galanin receptors," FEBS 446:103-107, 1999), endogenous ligands for this receptor have been isolated. These natural agonists, with a common theme of a RF-amide C terminus, derive from a precursor protein, kisspeptin-1 (Ohtaki et al., "Metasis suppressor gene Kiss-1 encodes peptide ligand of a G-protein-coupled receptor," Nature 411:613-617, 2001; Muir et al., "AXOR12, a novel human G protein-coupled receptor, activated by the peptide Kiss-1," J. Biol. Chem. 276:28969-28975, 2001; Kotani et al., "The metastasis suppressor gene Kiss-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54," J. Biol. Chem. 276:34631-34636, 2001). Kisspeptin-1 was originally isolated by differential display and subtractive hybridization from melanoma cells suppressed for their metastatic potential after microcell-mediated transfer of human chromosome 6 (Miele et al., "Metastasis suppressed, but tumorigenicity and local invasiveness unaffected, in the human melanoma cell line MelJuSo after introduction of human chromosomes 1 or 6," Mol. Carcinog. 15:284-299, 1996; Lee et al., "KiSS-1, a novel human malignant melanoma metastasis-suppressor gene," J. Natl. Cancer Inst. 88:1731-1737, 1996; Lee and Welch, "Identification of highly expressed genes in metastasis-suppressed chromosome 6/human malignant melanoma hybrid cells using subtractive hybridization and differential display," Int. J. Cancer 71:1035-1044, 1997), a chromosome preferentially lost during the progression of many melanomas (Trent et al., "Identification of a recurring translocation site involving chromosome 6 in human malignant melanoma," Cancer Res. 49:420-423, 1989; Walker et al., "Simple tandem repeat allelic deletions confirm the preferential loss of distal chromosome 6q in melanoma," Int. J. Cancer 58:203-206, 1994; Takata et al., "Clonal heterogeneity in sporadic melanomas as revealed by loss-of-heterozygosity analysis," Int. J. Cancer 85:492-497, 2000). When searching for natural agonists of the then "orphan" nuclear receptor GPR54, placental tissue extracts were discovered by multiple groups to demonstrate biological activity; when the active peptides were purified by mass

spectroscopy, they were found to be derived from kisspeptin-1 (Ohtaki et al., "Metasis suppressor gene Kiss-1 encodes peptide ligand of a G-protein-coupled receptor," Nature 411:613-617, 2001; Kotani et al., "The metastasis suppressor gene Kiss-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54," J. Biol. Chem. 276:34631-34636, 2001). The longest peptide (kisspeptin-1 68-121) is known as "mestastin" as predicted to result from proteolytic processing of the 121 amino acid parent protein. Metastin has recently been shown to be secreted from the placenta throughout gestation (Horikoshi et al., "Dramatic elevation of plasma metastin concentration in human pregnancy: metastin as a novel placenta-derived hormone in humans," J. Clin. Endocrinol. Metab. 88:914-919, 2003). Shorter C-terminal peptides (including kisspeptin-10 (amino acids 112-121); which is also referred to as metastin 45-54) share similar affinities and efficacies, and demonstrate that the C-terminal part of the peptide is responsible for the activation of GPR54. Other kisspeptin fragments that affect the activity of GPR54 include kisspeptin 94-121, 107-121, 108-121, 109-121, 113-121, and 114-121. (See e.g., Ohtaki et al., "Metasis suppressor gene Kiss-1 encodes peptide ligand of a G-protein-coupled receptor," Nature 411:613-617, 2001; Muir et al., "AXOR12, a novel human G protein-coupled receptor, activated by the peptide Kiss-1," J. Biol. Chem. 276:28969-28975, 2001; Kotani et al., "The metastasis suppressor gene Kiss-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54," J. Biol. Chem. 276:34631-34636, 2001.)

In addition, natural ligands for GPR54 may include members of the RFamide (neuropeptides terminating in -Arg-Phe-NH_2) and RWamide families (Clements et al., Biochem. Biophys. Res. Commun. 284:1189-1198 (2001).

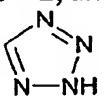
Small molecular weight agonists of GPR54 include those encompassed by one of the following formulas.

Formula I

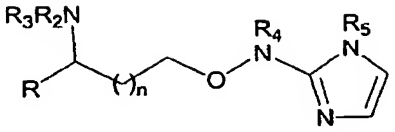


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

Z =

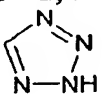


Formula II

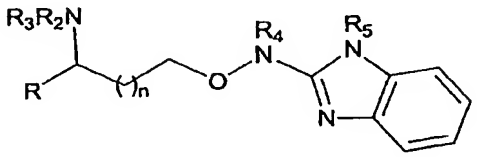


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

Z =

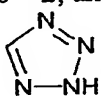


Formula III



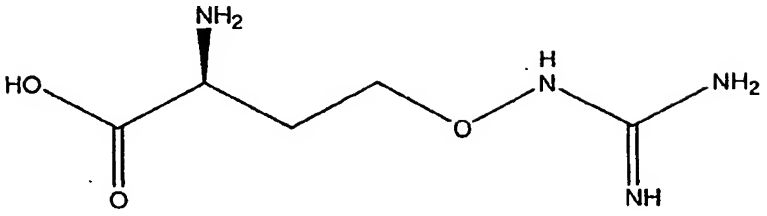
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

Z =



An exemplary small molecular weight agonist of GPR54 is LDN-21810, the

structure of which is shown below.



Kisspeptin regulates gonadotrophin secretion in the mouse

To determine whether metastin and kisspeptin-10 could stimulate LH release in adult male mice, metastin or kisspeptin-10 was administered via an intracerebroventricular (ICV) injection. In particular, the mice were handled daily for 2 weeks prior to the experiment. The mice were given an ICV injection of metastin (1 nmol) suspended in aCSF, kisspeptin-10 (1 nmol) suspended in artificial cerebrospinal fluid (aCSF) + 15% DMSO, aCSF + 15% DMSO alone or aCSF alone (n = 5 per group). Blood was obtained via orbital bleed 30 minute post-injection and sera were assayed for LH. These experiments showed that metastin and kisspeptin-10 stimulated LH secretion in the mouse ($p = 0.01$ vs. vehicle treated animals) (Fig. 2). LH was not different in those injected with aCSF or aCSF + 15% DMSO (data not shown).

To determine the lowest effective dose of metastin to stimulate LH secretion, metastin was given at varying doses. The mice were given an ICV injection of metastin in doses varying from 1 fmol to 5 nmol or aCSF alone (n = 5-8 per group). Doses administered include 1 fmol, 10 fmol, 0.1 pmol, 1 pmol, 10 pmol, 0.1 nmol, 0.375 nmol, 0.625 nmol, 1.25 nmol, 2.5 nmol, and 5nmol, and blood was obtained via orbital bleed 30 min post-injection and sera were assayed for LH. Clearly, metastin produced a significant increase in serum LH at all doses tested (Fig. 3). The responses to doses greater than 1 fmol were not significantly different from each other. The

response to 1 fmol was intermediate-significantly greater than vehicle ($p<0.05$) but less than the 10 fmol and higher doses ($p<0.001$).

To determine whether metastin's stimulatory effect on gonadotropin secretion was mediated by GnRH, we pretreated animals with acyline, a potent GnRH antagonist, before delivering the ICV injection of metastin. Mice received a subcutaneous injection of acyline (50 µg) dissolved in sterile saline (100 µl/mouse) 24 hours and 1 hour prior to ICV injection of metastin or aCSF alone. Control mice were given a subcutaneous injection of saline only at 24 hours and 1 hour prior to ICV injections. On the day of the experiment mice received either an ICV injection of metastin (0.05 nmol) or aCSF alone. Of the mice that had been given acyline, half were treated with metastin and the other half received aCSF (n = 6/group). Similarly, half of the animals that had been treated with saline were given metastin and the other half received aCSF (n = 6). Blood was obtained by orbital bleed 60 min post-ICV injection and sera were assayed for LH and FSH.

From these experiments, it is clear that metastin (0.05 nmol) significantly stimulated both LH ($p<0.0001$) and FSH secretion ($p<0.001$) compared to vehicle-treated animals. Gonadotropin responses to metastin were blocked in mice pretreated with acyline (metastin vs vehicle: $p>0.05$) (Fig. 4). Data from the acyline/vehicle treated group are not shown.

The distribution of *KiSS-1* mRNA in the hypothalamus of the mouse was also determined. Silver grain clusters, representing cells expressing KiSS-1 mRNA, were found at several levels through the rostral-caudal extent of the hypothalamus. Many cells expressing KiSS-1 mRNA were observed in the anteroventral periventricular nucleus (AVPV), the periventricular nucleus (PeN), and the arcuate nucleus (ARC) (Fig. 5). In the rostral aspect of the arcuate nucleus, KiSS-1 mRNA was found throughout both the medial and lateral divisions. However, in the caudal aspect of the arcuate nucleus, KiSS-1 mRNA expression was restricted to the ventral portion of the nucleus. Some cells expressing KiSS-1 mRNA were observed in the anterodorsal preoptic nucleus (ADP), whereas few cells were found in the medial amygdala and bed nucleus of the stria terminalis. (Muir and colleagues also report finding

significant expression of KiSS-1 in several other areas of the forebrain in the human, such as the caudate nucleus, globus pallidus, nucleus accumbens, putamen, and striatum (Muir et al., J. Biol. Chem 276:28969-28975, 2001).) The amount of KiSS-1 mRNA per cell, as estimated by the number of silver grains per cluster, did not appear to differ significantly among these anatomical regions of the hypothalamus. Including excess unlabeled antisense probe with radiolabeled antisense probe abolished all specific signal, and no signal was observed following the application of radiolabeled sense probe.

All data above are expressed as a mean ± SEM for each group. Differences among groups were assessed by one-way or two-way ANOVA. When the ANOVA indicated significant differences, Fisher's *post hoc* test was used to identify differences between individual treatment groups. Student's *t* test was used when only two groups were being compared. Differences were considered significant when $p < 0.05$.

The above data show that metastin delivered directly into the lateral cerebral ventricle stimulates LH and FSH secretion. This observation supports our finding that reproduction is regulated by a permissive kisspeptin/GPR54 pathway,

Administration of metastin to monkeys

To further support our finding that the metastin receptor can regulate GnRH release, metastin was administed to rhesus monkeys. Four juvenile (16.75 to 22.75 months of age and 2.9 to 3.9 kg body weight, at the time of initiation of experiments) orchidectomized rhesus monkeys (*Macaca mulatta*) were used for the *in vivo* study. The animals were bilaterally castrated 1.25 to 2.25 months before start of the experiments. The animals were housed in individual cages and maintained under a controlled photoperiod (lights on 0700-1900; 7 AM to 7 PM) and temperature (20°C), and fed daily a high protein monkey diet at approximately 1100 h (11 AM) supplemented with fruit in the afternoon. Drinking water was available *ad libitum*. (The animals were maintained according to the National Institutes of Health Guide for

Care and Use of Laboratory Animals and the protocols were approved by the Institutional Animal Care and Use Committee.)

The animals were implanted with an indwelling jugular catheter and fitted with a jacket and tether system (Suter et al., Endocrinology 139:2774-2783, 1998) for administration of an intermittent iv infusion of GnRH and for obtaining sequential blood samples. The animals were first subjected to a chronic pulsatile iv infusion of GnRH (0.3 μg every hour) for the purpose of enhancing the responsiveness of gonadotropes to GnRH stimulation to use pituitary LH secretion as a bioassay for endogenous release of GnRH (Suter et al., Endocrinology 139:2774-2783, 1998). Generally, it takes 3-4 weeks of pulsatile GnRH stimulation of the juvenile pituitary to increase plasma LH concentrations to levels approaching those observed in adults. Circulating LH levels fall abruptly to undetectable concentrations following cessation of the GnRH priming infusion. However, the response of the pituitary to GnRH is maintained for several days. In the present study, the GnRH priming infusion was stopped at least 3 days before testing the effect of kisspeptin-1, and subsequently re-initiated between the experiments to maintain pituitary responsiveness to GnRH.

To administer metastin centrally, monkeys were implanted with a 22-gauge stainless steel cannula in the lateral cerebroventricle (icv) 2-4 weeks before start of the experiments (Shahab et al., J. Neuroendorinol. 15:965-970, 2003). Orchidectomies and iv and ventricular cannulations were performed using standard aseptic surgical procedures.

Unrestrained and unsedated animals were administered vehicle (7.5% DMSO in aCSF), 30 μg metastin, and 100 μg metastin in 200 μl volume introduced slowly into the icv line via a 3-way connector above the swivel device and was immediately chased into the cerebroventricular (ICV) system with 450-570 μl aCSF: a volume in slight excess of the dead space of the icv catheters (Figures 1A and 1B). ICV challenges were given separately on 2 consecutive days while the third one was given at least 10 days later. On a separate occasion, animals were administered acyline subcutaneously (SC) (60 μg/kg in morning and 120 μg/kg in late afternoon) and the next day, were challenged with icv 100 μg metastin. All ICV injections were given

between 0900 to 0915 h (9 AM and 9:15 AM). Blood samples were obtained at 30 minute intervals for 30 minutes before ICV injections (-30 and 0 minutes) and for 240 minutes thereafter. On some occasions, animals were also administered an ICV bolus of 1229U91 an NPY receptor antagonist/agonist dissolved in aCSF (200 μg; GlaxoSmithKline, Research Triangle Park, NC) and an iv bolus of GnRH (0.3 μg) to confirm patency of the ICV cannula and responsivity of gonadotropes, respectively. Additional samples were collected 30 min post 1229U91 and 15 minute post GnRH.

Plasma LH concentrations were measured with a double antibody RIA system that employs recombinant cynomolgus LH (AFP342994) as standard and radioiodinated tracer, and a rabbit polyclonal antiserum to recombinant LH (AFP342994) as first antibody. The RIA reagents were provided by the National Hormone and Peptide Program. The sensitivity of the assay varied from 0.06 to 0.19 ng/ml and the intra- and inter-assay coefficients of variation were <3% and <6%, respectively.

The above experiments were carried out using the following materials and methods.

Peptides and reagents

Metastin (KiSS-1 (68-119)-NH_2 (mouse)/metastin (1-52)) was purchased from Phoenix Pharmaceutical, Belmont, CA. Human metastin (kisspeptin-1 112-121) was synthesized at the Peptide/Protein Core Facility of the Massachusetts General Hospital Endocrine/Reproductive Endocrine Unit. For experiments involving the administration of metastin to monkeys, metastin was dissolved in monkey artificial cerebrospinal fluid (aCSF; GIBCO BRL, Life Technologies, Grand Island, NY) containing 15% DMSO (Sigma Chemical Co., St. Louis, MO). The working metastin solution contained 7.5% DMSO. GnRH (GMP-26 code no. 230-110-40) and acyline were synthesized at the Salk institute (Contract N01-HD-0-2906) and Bioqual (Rockville, MD), respectively and made available by the Contraception and Reproductive Health Branch of the Center for Population Research at NICHD. GnRH was dissolved in saline and acyline was dissolved in 5% aqueous mannitol.

Example 2

Continuous Administration of Metastin 45-54 to Monkeys

As detailed below, we determined that a state of hypogonadotropism can be induced by continuous metastin 45-54 (kisspeptin-10) administration. In particular, we assessed the effect of continuous administration of metastin 45-54 on LH release in agonadal juvenile male monkeys. The experiment was initiated following confirmation that pituitary responsiveness to GnRH had been markedly upregulated by intermittent priming with synthetic GnRH. At this time (day 1), the iv intermittent infusion of GnRH was interrupted. One hour following the last priming pulse of GnRH, 10 μg of metastin 45-54 was administered as a bolus iv injection and 1 hour later the continuous iv infusion of metastin 45-54 (100 μg/h for 98 hours) was initiated. The volume of infusion was monitored on a daily basis. During the last 3 hours of the continuous metastin 45-54 infusion on day 5, the animals received, in sequence, a bolus injection of 10 μg metastin 45-54, a bolus injection of N-methyl-DL-aspartic acid (NMDA; 10 mg/kg body wt), and a bolus injection of GnRH. In one monkey, the bolus injection of metastin 45-54 was administered after the GnRH and NMDA challenge. One day after termination of the continuous metastin 45-54 infusion, the animals received another iv bolus of 10 μg of metastin 45-54. Finally, the intermittent priming infusion of GnRH was reinitiated.

Circulating concentrations of LH were monitored in blood samples collected on the following occasions: (a) on day 1 before and after the last GnRH priming pulse and the bolus of metastin 45-54 (during these times, series of blood samples were collected 10 minutes before and at 10, 20, 30, and 50 minutes after the peptide bolus; samples were also collected on day 1 during the first 12 hours of the continuous metastin 45-54 infusion (at 10, 20, 30, 50, 70, 90, 110, 130, 150, 170, 360 and 720 minutes into the infusion)); (b) on days 2, 3, and 4 of the continuous metastin 45-54 infusion, a single blood sample was collected in the morning and evening, at approximately 1000 hours (10 AM) and 2200 hours (10 PM), respectively; (in addition, in 2 of 3 animals, a nocturnal series of blood samples was collected on day 2 at 20 minute intervals over a 3 hour period (1900– 2200 hours; 7 PM – 10 PM); (c) on

day 5 of the continuous metastin 45-54 infusion, series of blood samples were collected to describe the LH response to bolus injections of metastin 45-54, NMDA, and GnRH; and (d) one day after termination of the continuous metastin 45-54 infusion, series of blood samples were collected before and after another iv bolus of metastin 45-54.

Non-heparinized blood samples were also collected in EDTA tubes before, during, and after the continuous metastin 45-54 infusion to measure metastin 45-54 levels in the circulation at a later date. Plasma samples were stored at –20°C or below.

After a 2-week interval, the control experiment was performed using a continuous infusion of vehicle (0.33% DMSO in sterile DPBS at 3 ml per hour for 98 hours).

Results

The last iv priming pulse of GnRH administered to 3 agonadal male monkeys at 0800 hours (8 AM) on day 1 induced an LH discharge that increased circulating LH levels from 4.2±0.7 ng/ml to a peak level of 5.8±0.1 ng/ml (mean±SEM; see Fig. 6). At 0900 hours (9 AM), the iv administration of 10 μg metastin also elicited a rise in plasma LH levels, the amplitude (basal to peak: 4.7±1.6 to 8.8±1.7 ng/ml) of which was almost 2-fold that produced by the preceding bolus of GnRH. At 1000 hours (10 AM), continuous exposure to metastin (100 μg/h) was initiated. Peak LH levels (10.6±0.8 ng/ml) were observed at 1–2 hours, and these then declined dramatically in the face of continuing exposure to metastin reaching within 12-hour values (approximately 1 ng/ml) indistinguishable from the control vehicle infusions. Interestingly, these very low LH levels were sustained during the day (1000 hours; 10 AM) but in the evening (2200 hours; 10 PM), modest, albeit non-significant, elevations were consistently observed during both continuous metastin and vehicle infusions (Fig. 7).

On day 5, single doses of metastin, NMDA and GnRH, were administered iv during the final 3 hours of the metastin infusion (Fig. 8). Although NMDA and GnRH elicited discharges of LH, metastin did not. Twenty-one hours after

termination of the metastin infusion, however, a profound LH response was induced by administration of an identical bolus of metastin. These results demonstrate adequate stores of releasable GnRH within GnRH neurons, adequate stores of LH in the gonadotrophs, and intact GnRH receptor signaling. Administration of 0.3μg "physiological" pulse of GnRH also elicited a robust LH response, again indicative of the retained signaling capacity of the GnRH receptor and intact LH stores. However, administration of a single bolus of metastin concomitant with continuous metastin infusion, at a dose that elicited an LH discharge at the beginning of the experiment, failed to evoke a LH response. These data suggest that the inability to maintain LH levels 6 hours after the initiation of the continuous metastin infusion is due to desensitization or down regulation of GPR54. Twenty-one hours after termination of the metastin infusion, a profound LH response was induced by administration of an identical bolus of metastin 45-54, indicating that sensitivity to exogenous metastin was restored twenty-one hours after the continuous metastin administration was stopped.

The above continuous administration experiments were carried our using the following reagents and methods.

Animals

Three juvenile male rhesus monkeys (*Macaca mulatta*, 19-20 months of age, 2.6-3.8 kg body weight) were used. The age of the animals at the end of the study was 21-23 months, and the pubertal reactivation of the hypothalamic-pituitary axis in this species occurs at around 36 months of age (Plant, T., "Puberty in Primates," *In:* Knobil and Neill (eds)., The Physiology of Reproduction, Raven Press, New York, 453-485, 2005). The animals were maintained under a controlled photoperiod (lights on between 0700-1900 h; 7 AM – 7 PM) and at approximately 21°C in accordance with the NIH guidelines for the Care and Use of Laboratory Animals. The experimental procedures were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

Surgical procedures

Bilateral castration and implantation of intravenous (iv) catheters (inner

diameter, 0.040 inches and outer diameter, 0.085 inches, Stuart Bio-Sil, Sil-Med Corporation, Taunton, MA) were performed under sterile conditions as described, for example, in Suter et al. ("The Pattern and Tempo of the Pubertal Reaugmentation of Open-Loop Pulsatile Gonadotropin-Releasing Hormone Release Assessed Indirectly in the Male Rhesus Monkey (*Macaca mulatta*)," Endocrinology 139:2774-2783, 1998). Briefly, the animals were sedated with ketamine hydrochloride (10-20 mg/kg body weight, intramuscularly (im), Ketaject, Phoenix Scientific Inc., St. Joseph, MO) and anesthetized by isoflurane inhalation (1-2%, in oxygen, Abbott Animal House, North Chicago, IL). Bilateral castration was performed a few weeks before or at the time of catheterization. Two indwelling catheters were employed; one placed in an internal jugular or subclavian vein and the other in a femoral vein. During the continuous infusion of metastin 45-54, one line was dedicated to infusion and one to sampling. The animals received a single intramuscular injection of penicillin (Pen-G, 40,000 U/kg body weight, Phoenix Scientific Inc., St. Joseph, MO) on the day of surgery. Post-surgically, the animals received twice daily intravenous injections of a broad-spectrum antibiotic (Kefzol, 25 mg/kg body weight, Apothecon, Princeton, NJ) and an analgesic (Ketofen, 2 mg/kg body weight, Fort Dodge Animal Health, Fort Dodge, IA) for 4 days. The routine maintenance of animals in remote sampling cages has been described, for example, in Suter et al. ("The Pattern and Tempo of the Pubertal Reaugmentation of Open-Loop Pulsatile Gonadotropin-Releasing Hormone Release Assessed Indirectly in the Male Rhesus Monkey (*Macaca mulatta*)," Endocrinology 139:2774-2783, 1998).

Collection of blood samples

Blood samples (1ml) were withdrawn via the sampling catheter into heparinized syringes, transferred to sterile tubes, and the plasma harvested after centrifugation. During periods of sequential sampling, packed blood cells were resuspended with sterile saline and returned to the respective animal. Plasma was stored at –20°C until required for assays.

In situ GnRH bioassay

To use pituitary LH secretion as a bioassay for endogenous GnRH release in

juvenile animals, the responsiveness of the gonadotropes to GnRH stimulation was first enhanced by a chronic pulsatile intravenous infusion of GnRH (0.15μg/minute for 2 minutes every hour) as described, for example, in Shahab et al. ("Increased Hypothalamic GPR54 Signaling: A Potential Mechanism for Initiation of Puberty in Primates," Proc. Natl. Acad. Sci. USA 102:2129-2134, 2005); Suter et al. ("The Pattern and Tempo of the Pubertal Reaugmentation of Open-Loop Pulsatile Gonadotropin-Releasing Hormone Release Assessed Indirectly in the Male Rhesus Monkey (*Macaca mulatta*)," Endocrinology 139:2774-2783, 1998); Gay and Plant ("Sustained Intermittent Release of Gonadotropin-Releasing Hormone in the Prepubertal Male Rhesus Monkey Induced by N-Methyl-DL-Aspartic Acid," Neuroendocrinology 48:147-152, 1988); and Shahab et al. ("Central Nervous System Receptors Involved in Mediating the Inhibitory Action of Neuropeptide Y on Luteinizing Hormone Secretion in the Male Rhesus Monkey (*Macaca mulatta*)," J. Neuroendocrinology 15:965-970, 2003). A robust, adult-like LH response to exogenous GnRH stimulation is usually established by ~3-4 weeks of pulsatile GnRH treatment (Shahab et al., "Central Nervous System Receptors Involved in Mediating the Inhibitory Action of Neuropeptide Y on Luteinizing Hormone Secretion in the Male Rhesus Monkey (*Macaca mulatta*)," J. Neuroendocrinology 15:965-970, 2003). Following termination of the priming infusion, circulating LH concentrations fall rapidly to undetectable levels, but the response of the pituitary to GnRH is maintained for several days allowing experimentally induced endogenous GnRH release to be easily detected. GnRH priming was reestablished between the metastin and vehicle infusions (Suter et al., "The Pattern and Tempo of the Pubertal Reaugmentation of Open-Loop Pulsatile Gonadotropin-Releasing Hormone Release Assessed Indirectly in the Male Rhesus Monkey (*Macaca mulatta*)," Endocrinology 139:2774-2783, 1998).

Gonadotropins

Plasma LH levels were measured using a homologous (macaque) radioimmunoassay as described, for example, in Ramaswamy et al. ("The Time Course of Follicle-Stimulating Hormone Suppression by Recombinant Human Inhibin

A in the Adult Male Rhesus Monkey (*Macaca mulatta*)," Endocrinology 139:3409-3415, 1998) and El Majdoubi et al. ("Effects of Orchidectomy on Levels of the mRNAs Encoding Gonadotropin-Releasing Hormone and other Hypothalamic Peptides in the Adult Male Rhesus Monkey (*Macaca mulatta*)," J. Neuroendocrinology 12:167-176, 2000). The sensitivity of the LH assays ranged between 0.36 – 0.42 ng/ml, and the intra- and inter-assay coefficients of variation for LH at 74% binding were ≤ 3.5 % and ≤ 13.6 %, respectively.

Peptides and reagents

Human metastin 45-54 was synthesized at the Peptide/Protein Core Facility of the Massachusetts General Hospital. A stock solution of the peptide (500μg/ml) was prepared in 5% dimethylsulfoxide (DMSO, Sigma Chemical Co., St. Louis, MO) in 0.9% NaCl (Abbott Laboratories, Chicago, IL) and stored at –80°C. For continuous administration, the metastin 45-54 infusate (100μg/3ml) was prepared the day before the experiment by diluting the stock preparation with sterile Dulbecco's PBS (DPBS without $CaCl_2$ and $MgSO_4$, Gibco BRL Products, Grand Island, NY) and stored at 4°C. During the experiment, the Buretrol containing the infusate was maintained at room temperature and re-filled every 24 hours. A stock 5% DMSO solution in sterile saline was prepared and stored at -80°C. For vehicle infusion, this stock was diluted 1:15 with sterile DPBS, stored at 4°C, and used as described for the metastin 45-54 infusate.

For bolus administration of metastin 45-54, a 10μg dose (10 μg/ml in sterile DPBS) was used. N-methy-DL-aspartic acid (NMDA, Sigma-Aldrich Inc., St. Louis, MO) was dissolved in sterile saline at a stock concentration of 50 mg/ml. On the days of NMDA administration, doses of 10 mg/kg body weight were prepared in 1 ml sterile saline and passed through a 0.22 μm filter (Fisher Scientific, Pittsburgh, PA) before injection. GnRH, synthesized at the Salk Institute (Contract N01-HD-0-2906), was obtained from the National Hormone and Peptide Program. A stock GnRH solution was prepared at 1 mg/ml in sterile saline and stored at -20°C. For intermittent infusion, GnRH was diluted to 0.30 μg/ml in saline, stored at -20°C, and used as required.

Example 3

Characterization of Kisspeptin and GPR54 Knockout Mice

Mice with targeted deletions of *Kiss1* and *Gpr54* were made by homologous recombination. For *Kiss1*, a restriction fragment encompassing exon 1 was replaced by a PGK Neo cassette. For Gpr54, a restriction fragment encompassing exon 2 was replaced.

Kiss1 -/- mice are hypogonadal. The *Kiss1* -/- males have shorter anogenital distance than +/+ animals (*Kiss1* -/- compared with +/+ $p < 0.05$) (Figure 9). The *Kiss1* -/- females have delayed time to vaginal opening when compared with wild type counterparts ($p< 0.05$) (Figure 10). The internal genitalia of the *Kiss1* -/- male and female animals are smaller than +/+ animals by gross inspection (Figures 11A and 11B). Testicular weight of the *Kiss1* -/- males is significantly smaller than that of +/+ males, $p< 0.05$ (Figure 12). Ovarian weight of the *Kiss1* -/- females is significantly smaller than that of +/+ females, $p< 0.05$ (Figure 13). Gonadotropin levels (both LH and FSH) are significantly lower in the *Kiss1* -/- male mice (Figures 14A and 14B). Similar patterns are observed for FSH in female mice. Gonadotropin levels in *Kiss1* -/- and *Kiss1* -/+ males rise robustly after injection with the C-terminal decapeptide of kisspeptin YNWNSFGLRY-amide (SEQ ID NO:2; Figure 15). As expected, LH levels do not rise in *Gpr54* -/- animals.

Example 4

Compounds that Alter GPR54 Biological Activity

Compounds that may be used to treat a reproductive disorder associated with altered GnRH secretion or action include compounds that bind a GPR54 polypeptide and that alter a biological activity of a GPR54 polypeptide. Such compounds may be used in the treatment of e.g., central precocious puberty, polycystic ovarian disease, a steroid hormone dependent cancer, ovarian cancer, preparation for *in vitro* fertilization, endometriosis, uterine fibroids, irregular periods, and infertility. In addition, the compounds may be used as contraceptives.

Exemplary compounds that bind a GPR54 polypeptide are mammalian kisspeptin polypeptides, such as human kisspeptin polypeptides. Desirably, human kisspeptin polypeptides contain a C-terminal fragment of the following amino acid sequence

MNSLVSWQLLLFLCATHFGEPLEKVASVGNSRPTGQQLESLGLLAPGEQSLPC TERKPAATARLSRRGTSLSPPPESSGSRQQPGLSAPHSRQIPAPQGAVLVQREK DLPNYNWNSFGLRFGKREAAPGNHGRSAGRGWGAGAGQ (SEQ ID NO:1). Such fragments may include amino acids 68-121, 94-121, 107-121, 108-121, 109-121, 112-121, 113-121, or 114-121 of the amino acid sequence of SEQ ID NO:1. Alternatively, a kisspeptin polypeptide may include amino acids 68-119 of the murine *KiSS-1* (GenBank Accession No. AF472576) gene product, or a fragment thereof.

Moreover, a kisspeptin polypeptide may be modified, for example, to increase its affinity for a GPR54 polypeptide or to increase its *in vivo* stability, while maintaining the ability to bind the GPR54 polypeptide. For instance, a kisspeptin polypeptide may be modified to bind a GPR54 polypeptide with a sufficiently high affinity or have an *in vivo* stability that, with intermittent administration, the concentration of the kisspeptin polypeptide maintained in a patient results the maintenance of an LH level that is below (for example, 3%, 5%, 8%, 10%, 12%, 15%, 20%, 25%, 30%, 50%, 70%, 80%, 90%, 95%, or even 100% below) the basal level observed prior to administration of the compound. Desirably, administration of the compound results in a desensitization of the GPR54 polypeptide to its natural ligand or a downregulation of the expression of the GPR54 polypeptide.

A derivative of kisspeptin polypeptide may include an amino acid substitution, such as the substitution of one amino acid for another within the same class (e.g., with a nonpolar, uncharged polar, charged polar, or phenyl R group), substitution of at least one L-amino acid for a D-amino acid, or substitution of a standard amino acid (i.e., an amino acid encoded by the genome of an organism) for non-standard amino acid or amino acid analog. Peptides where all L-amino acids have been substituted for D-amino acids are also desirable.

Amino acids with nonpolar R groups include: Alanine, Valine, Leucine,

Isoleucine, Proline, Phenylalanine, Tryptophan, and Methionine. Amino acids with uncharged polar R groups include: Glycine, Serine, Threonine, Cysteine, Tyrosine, Asparagine, and Glutamine. Amino acids with charged polar R groups (negatively charged at Ph 6.0) include: Aspartic acid and Glutamic acid. Basic amino acids (positively charged at pH 6.0) include: Lysine, Arginine, and Histidine (at pH 6.0). Amino acids with phenyl groups include: Phenylalanine, Trptophan, and Tyrosine. Desirable substitutions are: Lys for Arg and vice versa such that a positive charge may be maintained; Glu for Asp and vice versa such that a negative charge may be maintained; Ser for Thr such that a free -OH can be maintained; and Gln for Asn such that a free NH_2 can be maintained. In addition, a non-standard amino acid residue with non-amide linkages may be substituted for an amino acid at a given position.

Non-standard amino acids and amino acid analogs include, α,α -disubstituted amino acids, N-alkyl amino acids, lactic acids, C-α-methyl amino acids, β-methyl amino acids, β-alanine, norvaline, norleucine, 4-aminobutyric acid, orithine, hydroxyproline, sarcosine, citrulline, cysteic acid, cyclohexylalanine, 2-aminoisobutyric acid, 6-aminohexanoic acid, t-butylglycine, phenylglycine, o-phosphoserine, N-acetyl serine, N-formylmethionine, 3-methylhistidine, adamantylalanine, 3-benzothienylalanine, 4,4'-biphenylalanine, 3,3-diphenylalanine, homophenylalanine, 2,6-dichlorobenzyltyrosine, cyclohexyltyrosine, 7-benzyloxytryptophan, tri-tert-butyltryptophan, homotryptophan, 3-(-Anthracenyl)-L-alanine, L-p-iso-propylphenylalanine, L-thyroxine, and 3,3',5-triiodo-L-thyronine. These and numerous other amino acids and amino acid derivatives are commercially available from suppliers such as Novabiochem, Advanced ChemTech, Synthetech, and Neosystem Laboratoire (see, e.g., U.S. Patent Number 6,890,902).

In addition, to enhance stability, a kisspeptin polypeptide may be modified to be a cyclic peptide. Such a cyclic peptide may include an amino acid sequence encompassed by the formula $NH_2—X_{(n)}-Z-X_{(y)}—COOH$ and a cyclic bond between the Z residue and COOH other than a thioester bond, where X is an amino acid, an amino acid analog, a peptide mimetic, or a non-amide isostere, n is 0 to 55 and y is 1 to 55, and Z is an amino acid. These peptides may be synthesized using standard

methods in the art. In general, the functional groups of the constituent amino acids must be protected during the coupling reactions to avoid formation of undesired bonds. Exemplary protecting groups are described in Greene et al. ("Protective Groups in Organic Synthesis," John Wiley & Sons, New York 1991) and (The Peptides: Analysis, Synthesis, Biology, Vol. 3, Academic Press, New York 1981). For example, cyclic peptides may be synthesized using standard solid phase peptide synthesis using, e.g., a 2-chlorotrisyl chloride resin which does not impair the protective groups on various amino acid residues and from which the peptide can be separated with a weak acid. Peptide synthesis may be carried out, for example, according to the Fmoc (9-fluorenylmethoxycarbonyl) chemistry. For instance, an Fmoc-side chain-protected peptide-resin may be obtained by starting the synthesis from the C-terminus on a fully automated peptide synthesizer using Fmoc-side chain-protected amino acids. The protected peptide resin may be mixed with an acetic acid/trifluoroethanol/dichloromethane (1:1:8) mixture, and the resulting mixture may be filtered to separate the side-chain protected peptide liberated with the weak acid from the resin. The resultant peptide may be converted into a cyclic peptide using standard techniques, such as those described in U.S. Patent Number 6,943,233.

Kisspeptin polypeptides may further be modified in any manner that enhances *in vivo* stability or activity. Exemplary modifications include N-terminal acetylation, glycosylation, biotinylation, and the addition of a polyethylene glycol (PEG) molecule. For example, covalent attachment of PEG to peptides is a potentially useful approach for delivering water insoluble peptide drugs as shown by Felix (In Harris and Zalipsky, Eds., Poly(ethylene glycol) Chemistry and Biological Applications, A.C.S. Symposium Series 680, pp. 218-238, A.C.S. Washington, D.C., 1997). A PEG conjugate of a kisspeptin polypeptide can be prepared, for example, by using an appropriate PEGylated amino acid such as Fmoc-Ser(PEG)-OH or tBoc-Ser(PEG)-OH instead of the corresponding non-PEGylated amino acid during the solid-phase synthesis of the kisspeptin derivative. PEGylation of peptides is described, for example, in U.S. Patent Number 6,433,135.

Glycosylation of a kisspeptin polyeptide may involve the addition of an O-

linked oligosaccharide, which is attached to serine or threonine residues or an N-linked oligosaccharide, which is attached to asparagine residues in an Asn-X-Ser/Thr sequence, where X can be any amino acid except proline. Saccharide molecules may be linked to a peptide chain using, for example, a Diels-Alder reaction as described, for example, in U.S. Patent Number 6,958,395.

Moreover, the compounds used in the treatment methods described herein may be peptide mimetics of kisspeptin polypeptides. Such peptide mimetics are structurally similar to a kisspeptin polypeptide, but have one or more peptide linkages replaced by linkages such as $-CH_2NH-$, $-CH_2S-$, $-CH_2-CH_2-$, -CH=CH- (cis and trans), $-CH_2SO-$, $-CH(OH)CH_2-$, or $-COCH_2-$ by standard methods in the art (see, e.g., Dorner et al., Bioorganic and Med. Chem. 4:709-715, 1996).

Assays for characterizing compounds

Assays to be used for identifying and/or characterizing compounds, including small molecular weight agonists and antagonists of a GPR54 polypeptide, that alter a GPR54 biological activity may include measuring intracellular calcium release, phosphorylation of a polypeptide in a signaling pathway regulated by GPR54, arachidonic acid release, and phosphatidylinositol turnover. Further, the exemplary competitive binding assays described below may be used to identify a candidate compound that can be used in the claimed methods.

In addition, to derivatives of kisspeptin polypeptides, other compounds that bind a GPR54 polypeptide and that alter a biological activity of a GPR54 polypeptide may also be used in the treatment methods of the present invention. In general, compounds that alter a GPR54 biological activity can be identified from large libraries of both natural products, synthetic (or semi-synthetic) extracts or chemical libraries, according to methods known in the art.

Those skilled in the art will understand that the precise source of test extracts or compounds is not critical to the screening procedure(s) of the invention. Accordingly, virtually any number of chemical extracts or compounds can be screened using the exemplary methods described herein. Examples of such extracts or compounds include, but are not limited to, plant-, fungal-, prokaryotic- or animal-

based extracts, fermentation broths, and synthetic compounds, as well as modifications of existing compounds. Numerous methods are also available for generating random or directed synthesis (e.g., semi-synthesis or total synthesis) of any number of chemical compounds, including, but not limited to, saccharide-, lipid-, peptide-, and nucleic acid-based compounds. Synthetic compound libraries are commercially available from, for example, Brandon Associates (Merrimack, NH) and Aldrich Chemical (Milwaukee, WI).

Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are commercially available from a number of sources, including, but not limited to, Biotics (Sussex, UK), Xenova (Slough, UK), Harbor Branch Oceangraphics Institute (Ft. Pierce, FL), and PharmaMar, U.S.A. (Cambridge, MA). In addition, natural and synthetically produced libraries are produced, if desired, according to methods known in the art (e.g., by combinatorial chemistry methods or standard extraction and fractionation methods). Furthermore, if desired, any library or compound may be readily modified using standard chemical, physical, or biochemical methods.

In addition, those skilled in the art readily understand that methods for dereplication (e.g., taxonomic dereplication, biological dereplication, and chemical dereplication, or any combination thereof) or the elimination of replicates or repeats of materials already known for their effects on compounds associated with estrogen regulation should be employed whenever possible.

When a crude extract is found to alter a GPR54 biological activity, further fractionation of the positive lead extract is necessary to isolate chemical constituents responsible for the observed effect. Thus, the goal of the extraction, fractionation, and purification process is the careful characterization and identification of a chemical entity within the crude extract having activities that alter a GPR54 biological activity. The same *in vivo* and *in vitro* assays described herein for the detection of activities in mixtures of compounds can be used to purify the active component and to test derivatives thereof. Methods of fractionation and purification of such heterogenous extracts are known in the art.

GPR54 Binding Assays

A compound that binds GPR54 and/or competes with a known ligand for GPR54, for example a peptide derived from Kisspeptin (e.g., one including amino acids 68-121, 94-121, 107-121, 108-121, 109-121, 112-121, 113-121, or 114-121 of SEQ ID NO:1) or LDN-21810 or a derivative thereof is a candidate compound that may be used to treat a GPR54 related disorder. Such a compound may be identified by contacting a cell with a test compound in the presence of a kisspeptin polypeptide and assaying for a decrease of GnRH secretion or action, relative to a control cell in the presence of the kisspeptin polypeptide and not contacted with said test compound. A saturation-binding assay to determine the K_d value of the known ligand first may be performed. In these assays, the known ligand may be, for example, ^{125}I-kisspeptin-10 (amino acids 112-121 of SEQ ID NO:1). The known ligand can be used as a competitor for an unlabeled candidate compound. For instance, a reduction in ^{125}I-kisspeptin-10 binding in the presence of the candidate compound, when compared to the K_d value determined in a saturation binding experiment, is indicative that the candidate compound competitively binds GPR54. A compound that binds competitively to GPR54 may have a K_i of about, e.g., 1.0-5.0. Desirably, the K_i of a known ligand may be approximately 1.4, 1.6, 2.3, or 4.2.

In vitro protein binding assays are standard in the art and may include, e.g., the use of an epitope-tagged GPR54 polypeptide. Examples of commercially available epitope tags include, His-tags, HA-tags, FLAG®-tags, and c-Myc-tags. However, any epitope that is recognized by a polypeptide, such as an antibody, also may be used as an epitope tag. See, for example, Ausubel et al., *Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001; and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, N.Y., (1989). For instance, an epitope-tagged GPR54 protein used in *in vitro* binding experiments may be expressed in CHO cells. Polypeptides that bind GPR54 may be purified on protein gels and, further, may be identified by end-sequencing.

Furthermore, candidate compounds may be identified at the level of GPR54

polypeptide production using standard immunological techniques, such as Western blotting or immunoprecipitation with an antibody specific for a GPR54 polypeptide. For example, immunoassays may be used to detect or monitor the expression of a GPR54 polypeptide in an organism, tissue, or a cell line. Exemplary cell lines that may be used in these assays include GT1-7 (Mellon et al., Neuron 5:1-10, 1990); GnV-3 (Castillo et al., The Endocrine Society's 84th Annual Meeting, San Francisco, CA, Abstract OR45-2, 2002); and GN-11 (Zakaria et al., Mol. Endocrinol. 10:1282-1291, 1996). Polyclonal or monoclonal antibodies that are capable of binding to GPR54 may be used in any standard immunoassay format (e.g., ELISA, Western blot, or RIA assay) to measure the level of the polypeptide. In some embodiments, a compound that promotes an increase or decrease in the expression of a GPR54 polypeptide, or of a component of a GPR54 signaling pathway, is a candidate or test compound that may be used in the treatment or diagnosis of the reproductive disorders described herein, as well as in contraception and *in vitro* fertilization.

Alternatively, the expression of a GPR54 nucleic acid molecule may be measured, for example, by microarray analysis, Northern blot analysis (Ausubel et al., *Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001), or RT-PCR, using any appropriate fragment prepared from the nucleic acid molecule as a hybridization probe. The level of GPR54 nucleic acid molecule expression in the presence of the candidate compound may be compared to the level measured in a control culture medium lacking the candidate molecule. A compound which promotes an increase or a decrease in the expression of a GPR54 nucleic acid molecule or of a component of a GPR54 signaling pathway, is a candidate or test compound that may be used in the treatment or diagnosis of the reproductive disorders described herein, as well as in contraception and *in vitro* fertilization.

Moreover, a GPR54 nucleic acid molecule that includes a regulatory sequence may be expressed as a transcriptional or translational fusion with a detectable reporter, and expressed in a cell line. The cell expressing the fusion protein is then contacted with a candidate compound, and the expression of the detectable reporter in that cell is compared to the expression of the detectable reporter in an untreated control cell. A

candidate compound that alters (e.g., increases or decreases) the expression of the detectable reporter, e.g., luciferase, is a compound that may be useful for the diagnosis or treatment of a reproductive order described herein, a proliferative disorder, as well as in contraception and *in vitro* fertilization.

5

Example 5

Identification of a Small Molecule Weight Agonist of GPR54 Biological Activity

A small molecular weight agonist of GPR54 was identified using the IP-One assay described herein, in Figure 17, and as follows. The approach used to identify
10 the agonist of GPR54 biological activity can also be modified to identify antagonists of GPR54 biological activity.

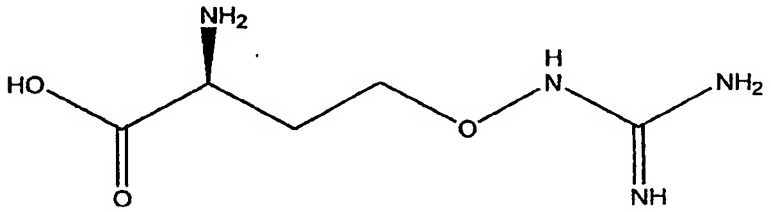
A stably transfected GPR54 expressing Chinese hamster ovary (CHO) cell line was generated. The vector expressing human GPR54 cDNA has the pIRESneo3 (Clontech) backbone (Figure 16A). pIRESneo3 is a bicistronic vector where the gene
15 of interest (GPR54) and the selection marker are under control of the same promoter. The use of the bicistronic promoter favors the selection of clones with a higher expression level of the transgene. As shown by Northern blot (Figure 16B), the GPR54 mRNA is highly expressed by the CHO cell line stably transfected with the GPR54 construct.

20 To measure the activation of the GPR54 receptor, an assay was developed that measures the amount of intracellular inositol monophosphate (IP1)- the IP-One assay (Figure 17). IP1 accumulation is a downstream signaling event that results from activation of the receptor. The assay is a competitive immunoassay with Europium cryptate-labeled antibodies to IP1 as the donor signal and d2 labeled IP1 as the
25 acceptor. When the cryptate and d2 fluorophores come in proximity to each other, there is an energy transfer and a signal results that can be measured on a standard plate reader. Results from kisspeptin stimulation of the receptor in this assay are shown in Figure 19. Figure 18 shows an assay of total intracellular inositol phosphates measured using myo-[2-3H]-inositol at the substrate.

30 A compound library of small molecules was screened in 384-well plates for

agonists to GPR54 with the stably transfected CHO cell line. This assay gave a Z' average of 0.76 (Figure 20A). The Z' factor is a measure of variability and when it is between 0.5 and 1.0 the assay is judged as a reproducible and robust assay suitable for high throughput screening. Controls included wells with DMSO for background signal and maximal concentration of Kisspeptin. Controls from representative plates are plotted in Figures 20B and 20C.

Compound LDN-21810 was identified from the screening, and activity, in a dose dependent manner, was confirmed in follow up assay measuring total inositol phosphate production with myo-[2-3H]-inositol as the substrate in the assay (Figure 21A). There was no activity with LDN-21810 in the cell line stably transfected with the empty vector (Figure 21B). The structure of LDN-21810 is shown below and in Figure 22.



Example 6

Biological Activity Assays

A compound that binds GPR54, or that alters the expression level of a GPR54 polypeptide or nucleic acid molecule, may be further screened to determine whether the compound alters a biological activity of GPR54, e.g., affects GnRH secretion or action. Such a compound may either decrease secretion of GnRH and cause hypogonadotropic hypogonadism as a therapy for various medical conditions such as sex steroid hormone dependent cancers, ovarian cancer, endometriosis, central precocious puberty, in preparation for *in vitro* fertilization, uterine fibroids, irregular periods, polycystic ovarian disease, and for contraception or restore normal pulsatile GnRH secretion in disorders in which it is abnormal. Compounds that may be used in the treatment methods of the present invention may be characterized using a number of standard methods in the art, including the following exemplary assays.

Intracellular Calcium Release Assay

An exemplary method to determine whether a compound alters a biological activity of a GPR54 polypeptide is an intracellular calcium release assay. In such assays mammalian cells, e.g., Chinese Hamster Ovary (CHO) cells, can be pretreated with 100 ng/ml pertussis toxin for 15 hours and incubated in Hanks' balanced salt medium containing 0.1% bovine serum albumin and 2.8 μg/ml Fura-2, a UV light-excitable, ratiometric Ca^{2+} indicator (Molecular Probes), at 37°C for 45 min in the presence or absence of a candidate compound. Upon binding Ca^{2+}, Fura-2 exhibits an absorption shift that can be observed by scanning the excitation spectrum between 300 and 400 nm, while monitoring the emission at ~510 nm. Fura-2 exhibits K_d values that are close to typical basal Ca^{2+} levels in mammalian cells (~100 nM), and displays high selectivity for Ca^{2+} binding relative to Mg^{2+} (see also, Kotani et al. (Br. J. Pharmacol. 133:138-144, 2001)). For this assay, the cells may be at a density of 10^5/ml and intracellular Ca^{2+} concentration can be measured using a luminescence spectrophotometer, e.g., a LS50B spectrophotometer (Perkin Elmer Life Sciences).

Phosphorylation Assays

Further, whether a compound alters a biological activity of a GPR54 polypeptide may be determined using assays that determine the phosphorylation state of proteins in a GPR54-regulated signaling pathway. For example, an alteration in the phosphorylation state of a downstream component of a GPR54-regulated signaling pathway is indicative of an alteration in the biological activity of a GPR54 polypeptide. Proteins that may be phosphorylated in a GPR54-regulated signaling pathway include focal adhesion kinase (FAK), paxillin, MAP kinases ERK1 and ERK2, PKA, PKC, and p38 MAP kinase (Kotani et al., Br. J. Pharmacol. 133:138-144, 2001; Kotani et al. J. Biol. Chem. 276:34631-34636, 2001). Phosphorylation assays may be carried out by plating a cell line (e.g., 4 x 10^6 CHO cells in Dulbecco's modified Eagle medium (DMEM) tissue culture medium) onto collagen-IV-coated 6 cm dishes and incubating these dishes for 2 hours at 37°C before contacting the cells with a compound that binds GPR54. The cells can then be lysed with 1 ml of lysis

buffer (50 mM Tris, 150 mM NaCl, 1 mM EGTA, 2 mM Na_3VO_4, 50 mM NaF, 1% NP-40, 4 mM $Na_4P_2O_7$ and protease inhibitors at pH 7.4). Each 0.45 ml of lysate can then be precipitated with an antibody against the potentially phosphorylated protein (e.g., FAK, paxillin, ERK1, ERK2, or p38 MAP kinase) using G-sepharose. One half of the precipitate may be Western blotted using ECL phosphorylation detection (Amersham Pharmacia), and the other half may be Western blotted with an antibody against the potentially phosphorylated protein.

Phosphatidylinositol Turnover

Monitoring phosphatidylinositol turnover may also be used to determine whether a compound alters a biological activity of a GPR54 polypeptide. For example, a mammalian cell line (e.g. CHO cells) may be cultured overnight in DMEM containing 5% fetal calf serum (FCS) and 1 μCi/ml *myo*-[^{3}H]inositol (Amersham Pharmacia Biotech). The cells can then be detached from the tissue culture plate with phosphate-buffered saline-EDTA, washed, and incubated for 20 minutes at 37°C with a candidate compound in 500 μl of 25 mM Tris-HCl, pH 7.4, 121.5 mM NaCl, 5.4 mM KCl, 0.8 mM $MgCl_2$, 1.8 mM $CaCl_2$, 15 mM glucose, and 10 mM LiCl. The incubation can be terminated with 500 μl of buffer containing 88% methanol and 0.12 M HCl. The cells may then be filtered through Whatman GF/B filters and washed with ice-cold buffer. Bound radioactivity can be measured in a TRI-CARB 2100TR counter (Packard Instrument Co.).

Arachidonic Acid Release Assay

One skilled in the art may also use an arachidonic release assay to determine whether a compound alters a biological activity of a GPR54 polypeptide. For instance, a mammalian cell line, e.g., CHO cells, may be cultured at a density of 2.5 x 10^6 cells/ml in 6-well plates overnight in 0.1 μCi/ml [^{3}H] arachidonic acid (Amersham Pharmacia Biotech), washed, and incubated for 60 minutes at 37°C with a candidate compound in DMEM containing 0.1% bovine serum albumin (BSA). The bound radioactivity can then be compared to a control which was incubated under the same

conditions, except in the absence of the candidate compound. Bound radioactivity may be measured, for example, using a TRI-CARB 2100TR counter (Packard Instrument Co.).

IP-One Assay

Monitoring inositol (1) phosphate (IP1) accumulation in a cell may also be used to measure the biological activity of a GPR54 polypeptide. Upon activation, G-Protein Coupled Receptors (GPCRs) carry information within the cell using two major signaling pathways depending on which G-protein is coupled: one results in variations of the cAMP level, whereas the other results in a transient increase of intracellular Ca^{2+} triggered by inositol (1,4,5) tri-phosphate (IP3). As the lifetime of IP3 is short, GPCR activation is better followed by monitoring IP3 degradation products, such as IP1, which accumulates in the cell in the presence of LiCl.

The IP-One assay uses HTRF® (Homogeneous Time Resolved Fluorescence). HTRF® is a technology based on TR-FRET, a combination of fluorescence resonance energy transfer (FRET) chemistry and the use of fluorophores with long emission half-lives. HTRF® uses a lanthanide with an extremely long half-life (Europium; "Eu^{3+}"). Eu^{3+} is conjugated to cryptate, an entity that confers increased assay stability. The assay also uses a monoclonal antibody specific to IP1, labeled with europium cryptate, competing with both native IP1 produced by cells and IP1 coupled with the dye d2 (an HTRF® acceptor). The specific signal (i.e., energy transfer) is inversely proportional to the concentration of IP1 in the calibrator or in the cell lysate.

The IP-One Assay uses lithium chloride (LiCl) 50 mM in the stimulation buffer to inhibit the phosphatase responsible for the degradation of IP1 into myo-inositol and, thereby, allows IP1 accumulation in the cell.

Within one hour after HTRF® reagent distribution, the signal reaches equilibrium and remains stable even after several days of incubation at 4°C. Moreover, the signal is robust and not light sensitive, so the same plate can be read several times without any alteration of the signal level.

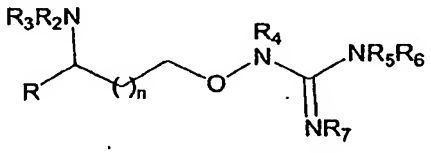
Using this assay, no cross-activity with 50 μM of (phosphor) inositide phosphates was observed.

Example 7

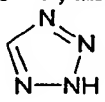
Administration of Compounds that Continuously Occupy a GPR54 Polypeptide or that Alter a GPR54 Biological Activity

The compounds described herein may be administered by any suitable means that result in a concentration that results in an alteration in GPR54 biological activity such as by the continuous occupation of a GPR54 polypeptide or interference (e.g., continuous interference) with a GPR54 biological activity upon reaching the target region. The following methods of administration are suitable for treating reproductive disorders, such as those described herein, as well as for contraception. Suitable compounds include antagonists which decrease the biological activity of a GPR54 polypeptide and agonists which can be used to desensitize GPR54 and, thereby, reduce its biological activity. Exemplary compounds include those encompassed by one of the following formulas.

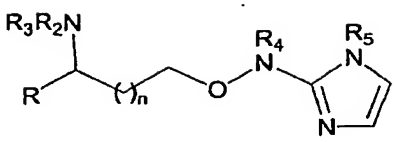
Formula I



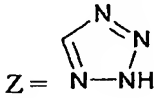
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

Z = 

Formula II

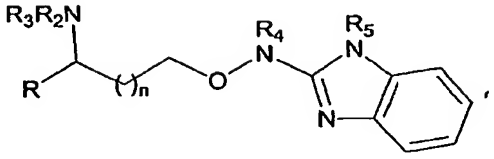


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

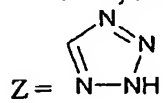


and

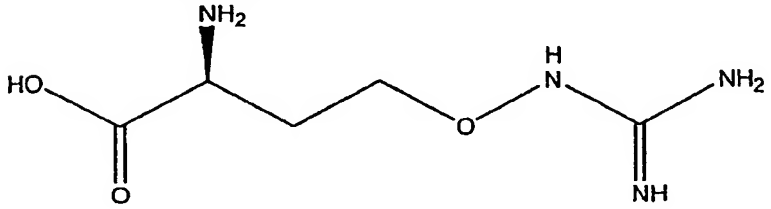
Formula III



where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



An exemplary small molecular weight agonist of GPR54 is



In the treatment methods described herein, a compound desirably binds a GPR54 polypeptide or a component of a GPR54 signaling pathway and decreases the luteinizing hormone (LH) level in a subject below the basal level observed in the subject prior to administration of the compound. Desirably the compound is administered continuously to a subject for at least four hours or intermittently over a period of at least four hours to decrease the LH level below the basal level. The decrease in LH level below the basal level desirably is at least 3%, 5%, 8%, 10%, 12%, 15%, 20%, 25%, 30%, 50%, 70%, 80%, 90%, 95%, or even 100% below the basal level. In another desirable embodiment, this decrease in the LH level is observed after the compound is administered continuously or intermittently for at least 5, 10, 12, 24, 36, 48, 60, 72, 84, or 96 hours. LH levels can be determined using standard techniques as described herein.

The therapeutic compound may be contained in any appropriate amount in any suitable carrier substance, and is generally present in an amount of 1-95% by weight of the total weight of the composition. The composition may be provided in a dosage form that is suitable for an oral (buccal), transdermal, transvaginal, or parenteral (e.g., subcutaneous, intravenous, intramuscular, or intraperitoneal) administration route. The pharmaceutical compositions may be formulated according to conventional pharmaceutical practice (see, e.g., Remington: The Science and Practice of Pharmacy (20th ed.), ed. A.R. Gennaro, Lippincott Williams & Wilkins, 2000 and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York). The pharmaceutical composition may be administered orally or parenterally by injection, infusion or implantation (subcutaneous, intravenous, intramuscular, intraperitoneal, or the like) in dosage forms, formulations, or via suitable delivery devices, such as a vaginal ring, or implants containing conventional, non-toxic pharmaceutically acceptable carriers, e.g., ethyl vinyl acetate, and adjuvants. The formulation and preparation of such compositions are well known to those skilled in the art of pharmaceutical formulation. Formulations can be found in Remington: The Science and Practice of Pharmacy, *supra*.

Formulations for oral use include tablets containing the active ingredient(s) in

a mixture with non-toxic pharmaceutically acceptable excipients, and such formulations are known to the skilled artisan (e.g., U.S. Patent Serial Nos.: 5,817,307, 5,824,300, 5,830,456, 5,846,526, 5,882,640, 5,910,304, 6,036,949, 6,036,949, 6,372,218, hereby incorporated by reference). These excipients may be, for example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose, glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, hydroxypropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants, and anti-adhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other pharmaceutically acceptable excipients can be, colorants, flavoring agents, plasticizers, humectants, buffering agents, and the like.

The tablets may be uncoated or they may be coated by known techniques, optionally to delay disintegration and absorption in the gastrointestinal tract and thereby providing a sustained action over a longer period. The coating may be adapted to release the active compound in a predetermined pattern (e.g., in order to achieve a controlled release formulation) or it may be adapted not to release the active compound until after passage of the stomach (enteric coating). The coating may be a sugar coating, a film coating (e.g., based on hydroxypropyl methylcellulose, methylcellulose, methyl hydroxyethylcellulose, hydroxypropylcellulose, carboxymethylcellulose, acrylate copolymers, polyethylene glycols and/or polyvinylpyrrolidone), or an enteric coating (e.g., based on methacrylic acid copolymer, cellulose acetate phthalate, hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, polyvinyl acetate phthalate, shellac, and/or ethylcellulose). Furthermore, a time delay material such as, e.g., glyceryl

monostearate or glyceryl distearate may be employed.

The solid tablet compositions may include a coating adapted to protect the composition from unwanted chemical changes, (e.g., chemical degradation prior to the release of the active compound). The coating may be applied on the solid dosage form in a similar manner as that described in Encyclopedia of Pharmaceutical Technology, supra.

Two active compounds may be mixed together in the tablet, or may be partitioned. In one example, the first active compound is contained on the inside of the tablet, and the second active compound is on the outside, such that a substantial portion of the second active compound is released prior to the release of the first active compound.

Formulations for oral use may also be presented as chewable tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent (e.g., potato starch, lactose, microcrystalline cellulose, calcium carbonate, calcium phosphate or kaolin), or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil. Powders and granulates may be prepared using the ingredients mentioned above under tablets and capsules in a conventional manner using, e.g., a mixer, a fluid bed apparatus, or spray drying equipment.

Controlled release compositions for oral use may, e.g., be constructed to release the active compound by controlling the dissolution and/or the diffusion of the active compound.

Dissolution or diffusion controlled release can be achieved by appropriate coating of a tablet, capsule, pellet, or granulate formulation of compounds, or by incorporating the compound into an appropriate matrix. A controlled release coating may include one or more of the coating substances mentioned above and/or, e.g., shellac, beeswax, glycowax, castor wax, carnauba wax, stearyl alcohol, glyceryl monostearate, glyceryl distearate, glycerol palmitostearate, ethylcellulose, acrylic resins, dl-polylactic acid, cellulose acetate butyrate, polyvinyl chloride, polyvinyl acetate, vinyl pyrrolidone, polyethylene, polymethacrylate, methylmethacrylate, 2-

hydroxymethacrylate, methacrylate hydrogels, 1,3 butylene glycol, ethylene glycol methacrylate, and/or polyethylene glycols. In a controlled release matrix formulation, the matrix material may also include, e.g., hydrated metylcellulose, carnauba wax and stearyl alcohol, carbopol 934, silicone, glyceryl tristearate, methyl acrylate-methyl methacrylate, polyvinyl chloride, polyethylene, and/or halogenated fluorocarbon.

A controlled release composition containing one or more of the compounds identified using the claimed methods may also be in the form of a buoyant tablet or capsule (i.e., a tablet or capsule that, upon oral administration, floats on top of the gastric content for a certain period of time). A buoyant tablet formulation of the compound(s) can be prepared by granulating a mixture of the active compound with excipients and 20-75% w/w of hydrocolloids, such as hydroxyethylcellulose, hydroxypropylcellulose, or hydroxypropylmethylcellulose. The obtained granules can then be compressed into tablets. On contact with the gastric juice, the tablet forms a substantially water-impermeable gel barrier around its surface. This gel barrier takes part in maintaining a density of less than one, thereby allowing the tablet to remain buoyant in the gastric juice.

With respect to the therapeutically active compounds identified using the methods of the invention, it is not intended that the administration of the claimed compound to a patient be limited to a particular mode of administration, dosage, or frequency of dosing; the present invention contemplates all modes of administration, including oral, intramuscular, intravenous, intraperitoneal, intravesicular, intraarticular, intralesional, subcutaneous, transvaginal, or buccal or any other route sufficient to provide a dose adequate to alter a biological activity of a GPR54 polypeptide. The compound(s) may be administered to the patient continuously or in multiple doses. For example, the compound may be continuously administered for at least 10, 12, 24, 36, 48, 60, 72, 84, or 96 hours. However, continuous administration may span 1, 2, or 3 weeks, 1, 2, 3, 4, 5, 6, 9, or 12 months, or even 2, 3, or more years. Moreover, the compound may also be continuously administered until the subject is free of the disease being treated or until contraception or preparation for *in vitro* fertilization is no longer indicated.

Alternatively, a compound may be intermittently administered in at least two doses over a span of at least four hours. Desirably the compound is intermittently administered once every 1, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 72, or 96 or more hours. The intermittent administration interval desirably is repeated for 10, 12, 24, 36, 48, 60, 72, 84, or 96 or more hours. However, the intermittent administration interval may be repeated for 1, 2, or 3 weeks, 1, 2, 3, 4, 5, 6, 9, or 12 months, or even several years. The compound may also be intermittently administered until the subject is free of the disease being treated or until contraception or preparation for *in vitro* fertilization is no longer indicated. The period between doses is generally determined by the *in vivo* stability of the compound, which, in turn, determines whether the LH level is maintained below the basal level observed before the first administration of the compound. It is to be understood that, for any particular subject, specific dosage regimes should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. The precise dose will vary dependent on the compound used and the rate of clearance of the polypeptide.

While the attending physician ultimately will decide the appropriate amount and dosage regimen, a therapeutically effective amount of a compound identified using the claimed method may be, for example, in the range of about 0.1 mg to 50 mg/kg body weight/day or 0.70 mg to 350 mg/kg body weight/week. Desirably a therapeutically effective amount is in the range of about 0.10 mg to 20.0 mg/kg, and more desirably in the range of about 0.20 mg to 15.0 mg/kg for example, about 0.2, 0.3, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 8.0, 8.5, 9.0, 10.0, 11.0, 12.0, 13.0, 14.0, or 15.0 mg/kg body weight administered daily, every other day, or twice a week.

For example, a suitable dose is an amount of the therapeutic compound that, when administered as described above, is capable of decreasing the biological activity of a GPR54 polypeptide by at least 20% below the basal (i.e., untreated) level. In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic

benefit. Such a response can be monitored by establishing an improved clinical outcome (e.g., more frequent remissions, complete or partial, or longer disease-free survival) in treated patients as compared to non-treated patients. According to this invention, the administration of the a compound described herein can decrease a biological activity of a GPR54 polypeptide by at least 20%, 40%, 50%, or 75% below that of an untreated control. Such responses can be monitored by any standard technique known in the art including those described herein, such as calcium release assays, determining the phosphorylation state of a polypeptide in a signaling pathway regulated by GPR54, arachidonic acid release assays, and phosphatidylinositol turnover assays. More preferably, the biological activity of a GPR54 polypeptide is decreased by 80%, 90%, 95%, or even 100% below that of an untreated control.

Other Embodiments

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth.

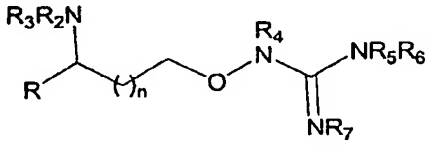
Seminara et al. (N. Engl. J. Med. 349:1614-1627, 2003), WO 2004/101747, and all other references, patents, patent application publications, and patent applications cited herein are hereby incorporated by reference to the same extent as if each of these references, patents, patent application publications, and patent applications were separately incorporated by reference herein.

What is claimed is:

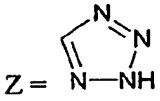
CLAIMS

1. A method for treating a reproductive disorder, said method comprising administering to a patient in need thereof an effective amount of a compound that alters a GPR54 biological activity, wherein said compound is selected from the group consisting of

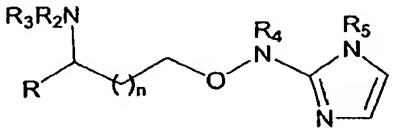
Formula I



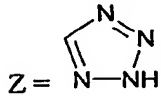
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



Formula II

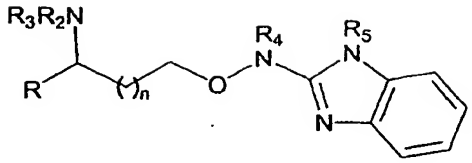


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

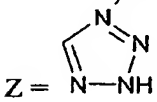


and

Formula III



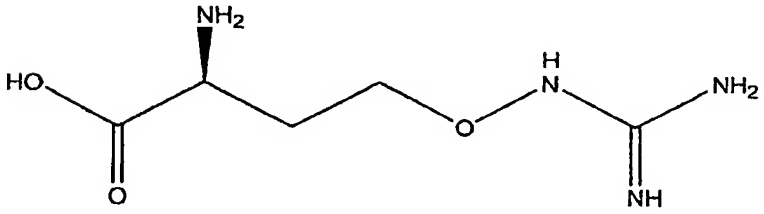
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



wherein said administering alters said GPR54 biological activity.

2. The method of claim 1, wherein said administering decreases said GPR54 biological activity.

3. The method of claim 1, wherein said compound is:



4. A method for treating a reproductive disorder, said method comprising administering to a patient in need thereof an effective amount of a compound that continuously interferes with a GPR54 biological activity, wherein said administering decreases said GPR54 biological activity.

5. A method for treating a reproductive disorder, said method comprising administering to a patient in need thereof an effective amount of a compound that

specifically binds to a GPR54 polypeptide, wherein said compound continuously occupies the GPR54 polypeptide, and wherein said administering decreases a biological activity of the GPR54 polypeptide.

6. A method for treating a reproductive disorder, said method comprising administering to a patient in need thereof an effective amount of a compound that specifically binds to a GPR54 polypeptide, wherein said administration results in a luteinizing hormone level in said patient that is below a basal luteinizing hormone level observed in said patient prior to said administration.

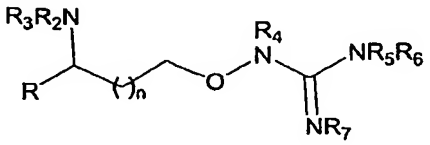
7. The method of claim 4, 5, or 6, wherein said compound is a peptide, a peptide derivative, or a small molecular weight agonist or antagonist of said GPR54 polypeptide.

8. The method of claim 4, wherein the continuous interference with the GPR54 biological activity comprises continuous administration of an effective amount of the compound to the patient.

9. The method of claim 5, wherein said continuously occupying the GPR54 polypeptide comprises continuous administration of an effective amount of the compound to the patient.

10. The method of claim 4, 5, or 6, wherein said compound is selected from the group consisting of

Formula I

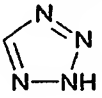


where

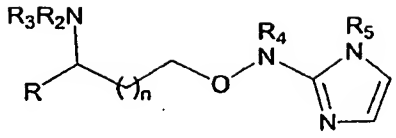
R is CO_2R_1 or Z;

R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;

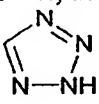
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

Z = 

Formula II

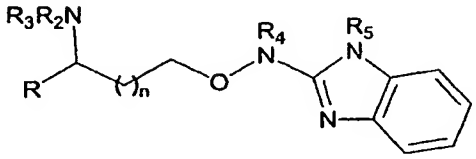


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

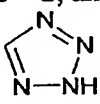
Z = 

and

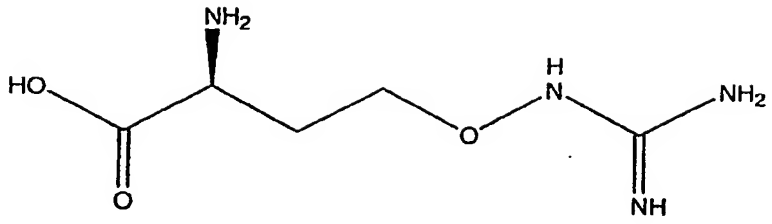
Formula III



where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

Z = 

11. The method of claim 10, wherein said compound is:



12. The method of claim 4, 5, or 6, wherein said compound is a kisspeptin polypeptide or a derivative thereof.

13. The method of claim 12, wherein said kisspeptin polypeptide comprises amino acids 68-121, 94-121, 107-121, 108-121, 109-121, 112-121, 113-121, or 114-121 of SEQ ID NO:1.

14. The method of claim 13, wherein said kisspeptin polypeptide consists of amino acids 112-121 of SEQ ID NO:1.

15. The method of claim 1, 4, 5, or 6, wherein said reproductive disorder is selected from the group consisting of central precocious puberty, polycystic ovarian disease, endometriosis, irregular periods, and uterine fibroids.

16. The method of claim 1, 4, 5, or 6, wherein said administering reduces the level of GnRH, a gonadotropin, or a sex steroid in said patient.

17. The method of claim 1, 4, 5, or 6, wherein said administering induces a state of hypogonadotropic hypogonadism in said patient.

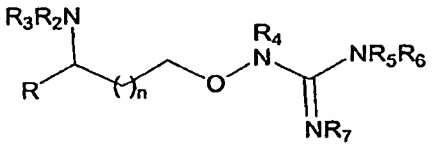
18. The method of claim 1, 4, 5, or 6, wherein said administering comprises intramuscular, intravenous, subcutaneous, transdermal, transvaginal, or oral administration.

19. The method of claim 18, wherein said transvaginal administration

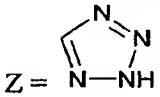
comprises a vaginal ring.

20. A method for treating a proliferative disorder, said method comprising administering to a patient in need thereof an effective amount of a compound that alters a GPR54 biological activity, wherein said compound is selected from the group consisting of

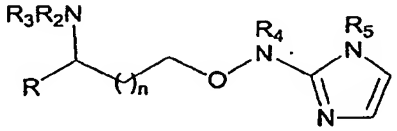
Formula I



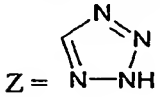
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



Formula II

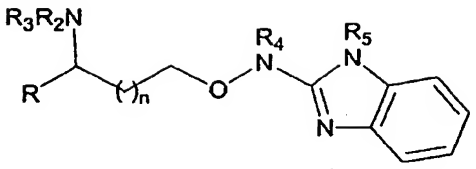


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

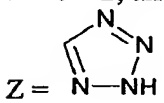


and

Formula III



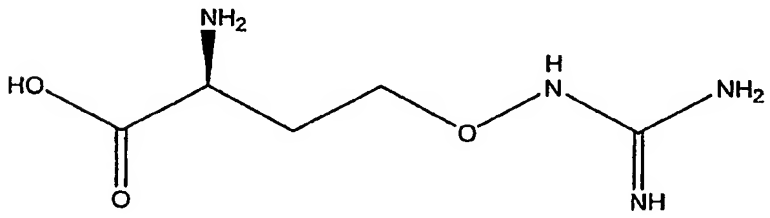
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



wherein said administering alters said GPR54 biological activity.

21. The method of claim 20, wherein said administering decreases said GPR54 biological activity.

22. The method of claim 20, wherein said compound is:



23. A method for treating a proliferative disorder, said method comprising administering to a patient in need thereof an effective amount of a compound that continuously interferes with a GPR54 biological activity, wherein said administering decreases said GPR54 biological activity.

24. A method for treating a proliferative disorder, said method comprising administering to a patient in need thereof an effective amount of a compound that specifically binds to a GPR54 polypeptide, wherein said compound continuously occupies the GPR54 polypeptide, and wherein said administering decreases a biological activity of the GPR54 polypeptide.

25. A method for treating a proliferative disorder, said method comprising administering to a patient in need thereof an effective amount of a compound that specifically binds to a GPR54 polypeptide, wherein said administration results in a luteinizing hormone level in said patient that is below a basal luteinizing hormone level observed in said patient prior to said administration

26. The method of claim 20, 23, 24, or 25, wherein said proliferative disorder is selected from the group consisting of prostate cancer, breast cancer, uterine cancer, ovarian cancer, and testicular cancer.

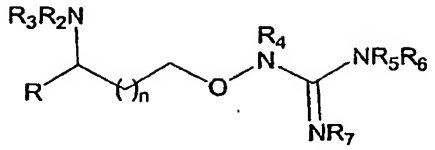
27. The method of claim 23, 24, or 25, wherein said compound is a peptide, a peptide derivative, or a small molecular weight agonist or antagonist of said GPR54 polypeptide.

28. The method of claim 23, wherein said continuous interference with the GPR54 biological activity comprises continuous administration of an effective amount of the compound to the patient.

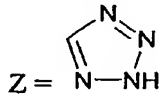
29. The method of claim 24, wherein the continuously occupying the GPR54 polypeptide comprises continuous administration of an effective amount of the compound to the patient.

30. The method of claim 23, 24, or 25, wherein said compound is selected from the group consisting of

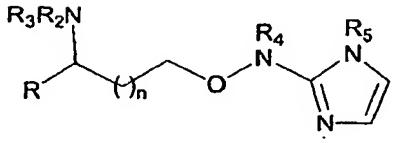
Formula I



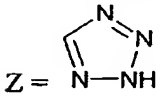
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



Formula II

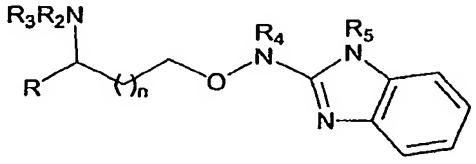


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

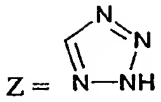


and

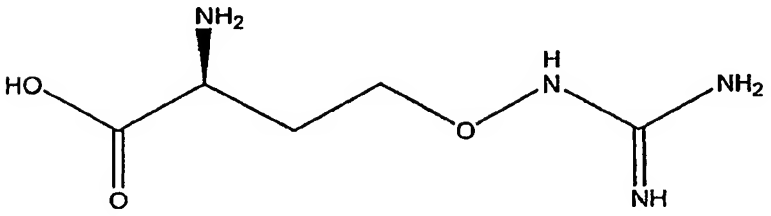
Formula III



where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



31. The method of claim 30, wherein said compound is:



32. The method of claim 23, 24, or 25, wherein said compound is a kisspeptin polypeptide or a derivative thereof.

33. The method of claim 32, wherein said kisspeptin polypeptide comprises amino acids 68-121, 94-121, 107-121, 108-121, 109-121, 112-121, 113-121, or 114-121 of SEQ ID NO:1.

34. The method of claim 33, wherein said kisspeptin polypeptide consists of amino acids 112-121 of SEQ ID NO:1.

35. The method of claim 20, 23, 24, or 25, wherein said administering reduces the level of GnRH, a gonadotropin, or a sex steroid in said patient.

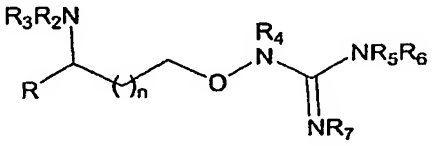
36. The method of claim 20, 23, 24, or 25, wherein said administering comprises intramuscular, intravenous, subcutaneous, transdermal, transvaginal, or oral administration.

37. The method of claim 36, wherein said transvaginal administration

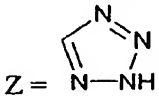
comprises a vaginal ring.

38. A method for contraception in a patient, said method comprising administering to a patient in need thereof an effective amount of a compound that alters a GPR54 biological activity, wherein said compound is selected from the group consisting of

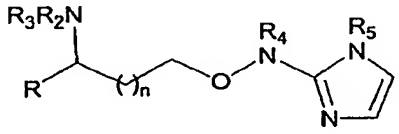
Formula I



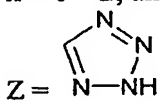
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



Formula II

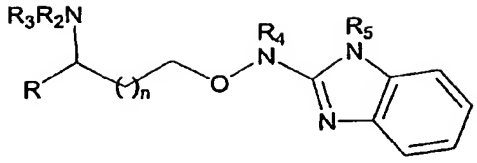


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

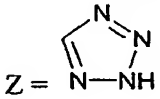


and

Formula III



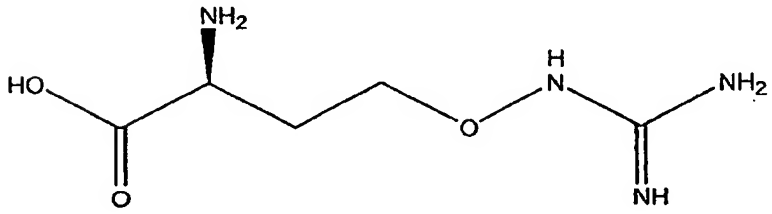
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



wherein said administering alters said GPR54 biological activity.

39. The method of claim 38, wherein said administering decreases said GPR54 biological activity.

40. The method of claim 38, wherein said compound is:



41. A method for contraception in a patient, said method comprising administering to a patient in need thereof an effective amount of a compound that continuously interferes with a GPR54 biological activity, wherein said administering decreases said GPR54 biological activity.

42. A method for contraception in a patient, said method comprising

administering to a patient in need thereof an effective amount of a compound that specifically binds to a GPR54 polypeptide, wherein said compound continuously occupies the GPR54 polypeptide, and wherein said administering decreases a biological activity of the GPR54 polypeptide.

43. A method for contraception in a patient, said method comprising administering to a patient in need thereof an effective amount of a compound that specifically binds to a GPR54 polypeptide, wherein said administration results in a luteinizing hormone level in said patient that is below a basal luteinizing hormone level observed in said patient prior to said administration

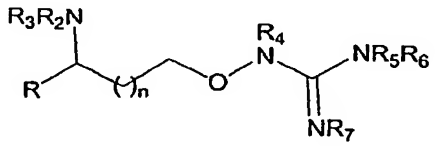
44. The method of claim 41, 42, or 43, wherein said compound is a peptide, a peptide derivative, or a small molecular weight agonist or antagonist of said GPR54 polypeptide.

45. The method of claim 42, wherein the continuous interference with the GPR54 biological activity comprises continuous administration of an effective amount of the compound to the patient.

46. The method of claim 43, wherein said continuously occupying the GPR54 polypeptide comprises continuous administration of an effective amount of the compound to the patient.

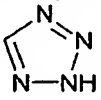
47. The method of claim 41, 42, or 43, wherein said compound is selected from the group consisting of

Formula I

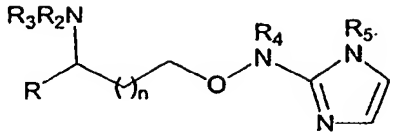


where

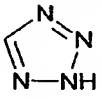
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

Z = 

Formula II

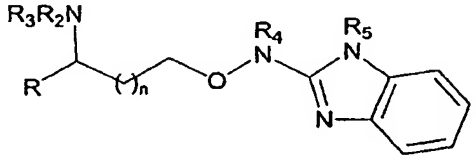


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

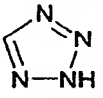
Z = 

and

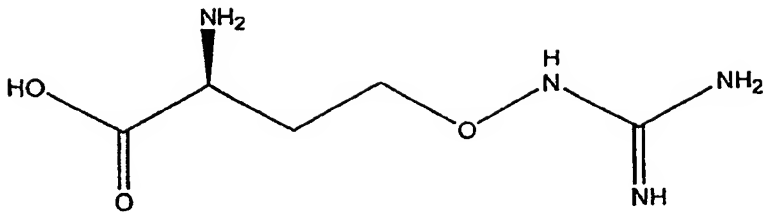
Formula III



where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

Z = 

48. The method of claim 47, wherein said compound is:



49. The method of claim 41, 42, or 43; wherein said compound is a kisspeptin polypeptide or a derivative thereof.

50. The method of claim 49, wherein said kisspeptin polypeptide comprises amino acids 68-121, 94-121, 107-121, 108-121, 109-121, 112-121, 113-121, or 114-121 of SEQ ID NO:1.

51. The method of claim 50, wherein said kisspeptin polypeptide consists of amino acids 112-121 of SEQ ID NO:1.

52. The method of claim 38, 41, 42, or 43, wherein said administering reduces the level of GnRH, a gonadotropin, or sex steroid in said patient.

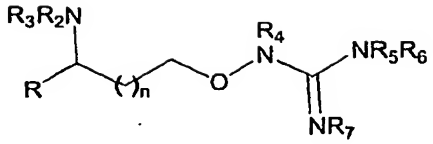
53. The method of claim 38, 41, 42, or 43, wherein said administering comprises intramuscular, intravenous, subcutaneous, transdermal, transvaginal, or oral administration.

54. The method of claim 53, wherein said transvaginal administration comprises a vaginal ring.

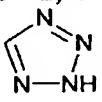
55. A method for treating infertility in a patient, said method comprising administering to a patient in need thereof an effective amount of a compound that alters a GPR54 biological activity, wherein said compound is selected from the group

consisting of

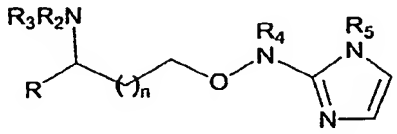
Formula I



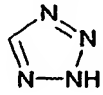
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

Z = 

Formula II

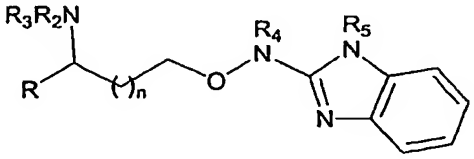


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

Z = 

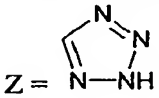
and

Formula III



where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;

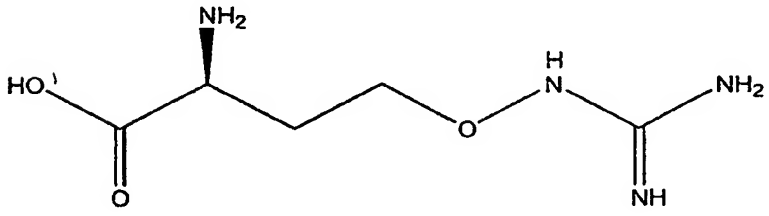
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



wherein said administering alters said GPR54 biological activity.

56. The method of claim 55, wherein said administering decreases said GPR54 biological activity.

57. The method of claim 55, wherein said compound is:



58. A method for treating infertility in a patient, said method comprising administering to a patient in need thereof an effective amount of a compound that continuously interferes with a GPR54 biological activity, wherein said administering decreases said GPR54 biological activity.

59. A method for treating infertility in a patient, said method comprising administering to a patient in need thereof an effective amount of a compound that specifically binds to a GPR54 polypeptide, wherein said compound continuously occupies the GPR54 polypeptide, and wherein said administering decreases a biological activity of the GPR54 polypeptide.

60. A method for treating infertility in a patient, said method comprising administering to a patient in need thereof an effective amount of a compound that

specifically binds to a GPR54 polypeptide, wherein said administration results in a luteinizing hormone level in said patient that is below a basal luteinizing hormone level observed in said patient prior to said administration

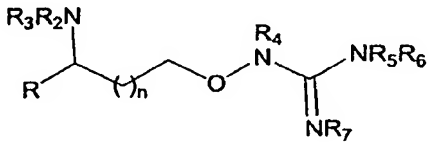
61. The method of claim 58, 59, or 60, wherein said compound is a peptide, a peptide derivative, or a small molecular weight agonist or antagonist of said GPR54 polypeptide.

62. The method of claim 58, wherein the continuous interference with the GPR54 biological activity comprises continuous administration of an effective amount of the compound to the patient.

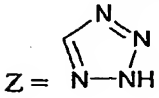
63. The method of claim 59, wherein said continuously occupying the GPR54 polypeptide comprises continuous administration of an effective amount of the compound to the patient.

64. The method of claim 58, 59, or 60, wherein said compound is selected from the group consisting of

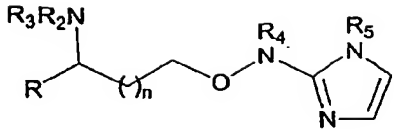
Formula I



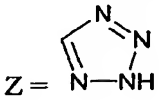
where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, R_5, R_6, and R_7 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



Formula II

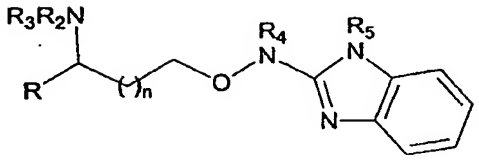


where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and

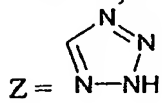


and

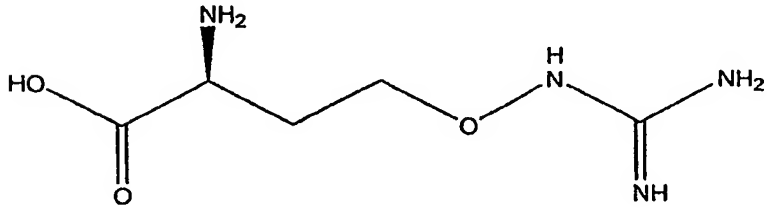
Formula III



where
R is CO_2R_1 or Z;
R_1, R_2, R_3, R_4, and R_5 is H, lower alkyl, aryl, or heteroaryl;
R_2 and R_3 is C(=O)Me;
n = 0 – 2; and



65. The method of claim 64, wherein said compound is:



66. The method of claim 58, 59, or 60, wherein said compound is a kisspeptin polypeptide or a derivative thereof.

67. The method of claim 66, wherein said kisspeptin polypeptide comprises amino acids 68-121, 94-121, 107-121, 108-121, 112-121, or 114-121 of SEQ ID NO:1.

68. The method of claim 67, wherein said kisspeptin polypeptide consists of amino acids 112-121 of SEQ ID NO:1.

69. The method of claim 55, 58, 59, or 60, wherein said administering reduces the level of GnRH, a gonadotropin, or a sex steroid in said patient.

70. The method of claim 55, 58, 59, or 60, wherein said administering comprises intramuscular, intravenous, subcutaneous, transdermal, transvaginal, or oral administration.

71. The method of claim 70, wherein said transvaginal administration comprises a vaginal ring.

72. The method of any one of claims 1, 4, 5, 6, 20, 23, 24, 25, 38, 41, 42, 43, 55, 58, 59, or 60, wherein said patient is a human.

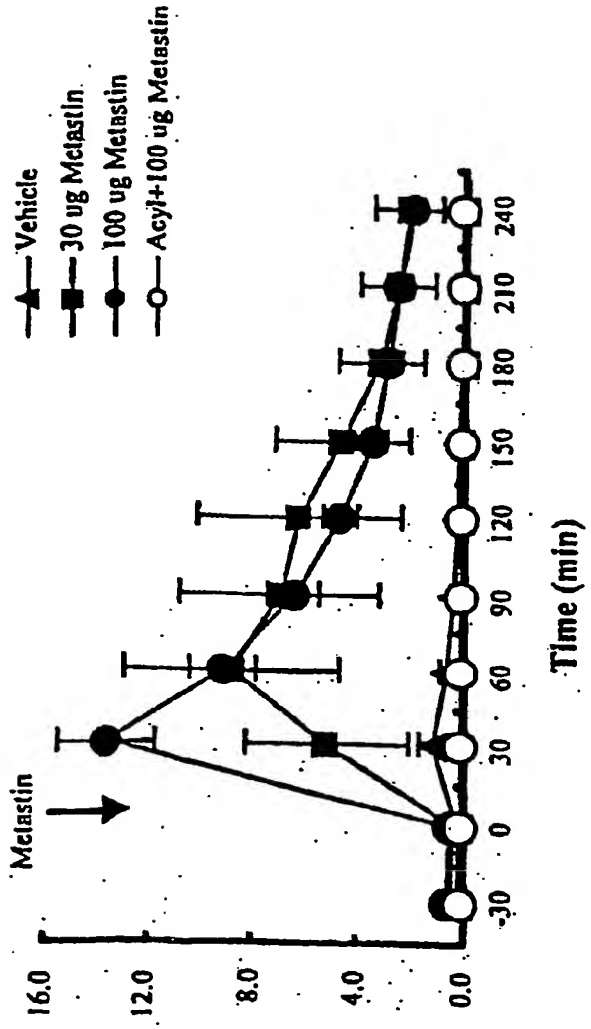

Vehicle
30 ug Metastin
100 ug Metastin
Acyl+100 ug Metastin
Metastin
16.0
12.0
8.0
4.0
0.0
-30
0
30
60
90
120
150
180
210
240
Time (min)

FIGURE 1A

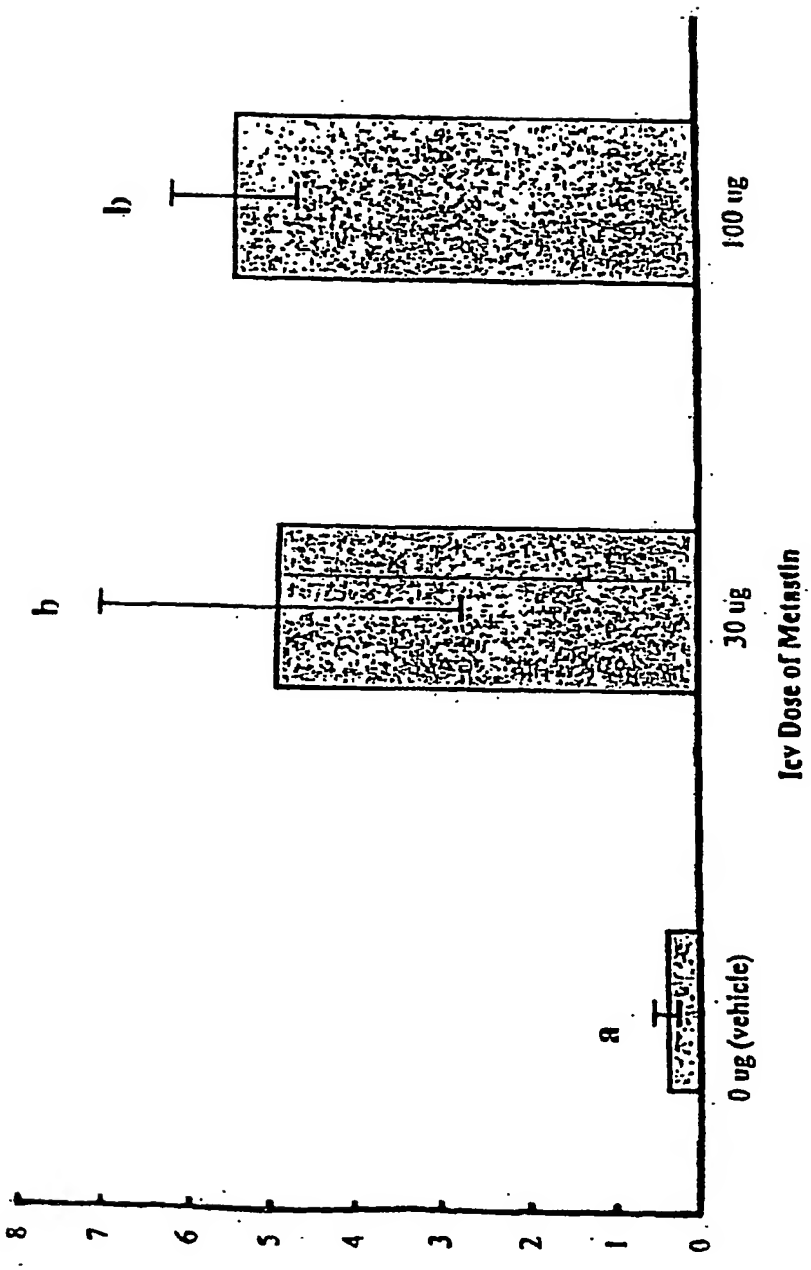


FIGURE 1B

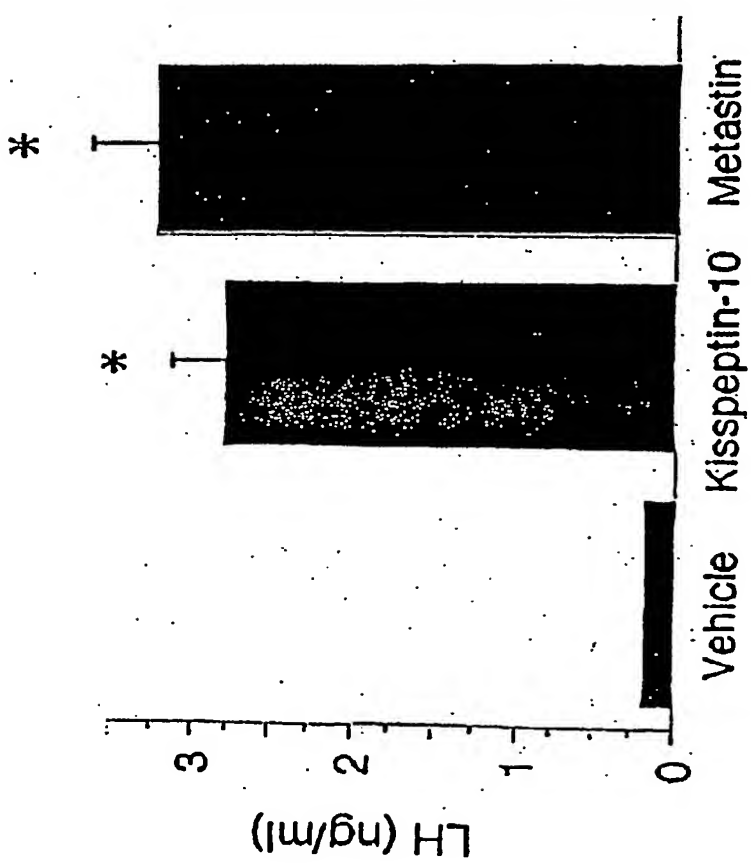


FIGURE 2

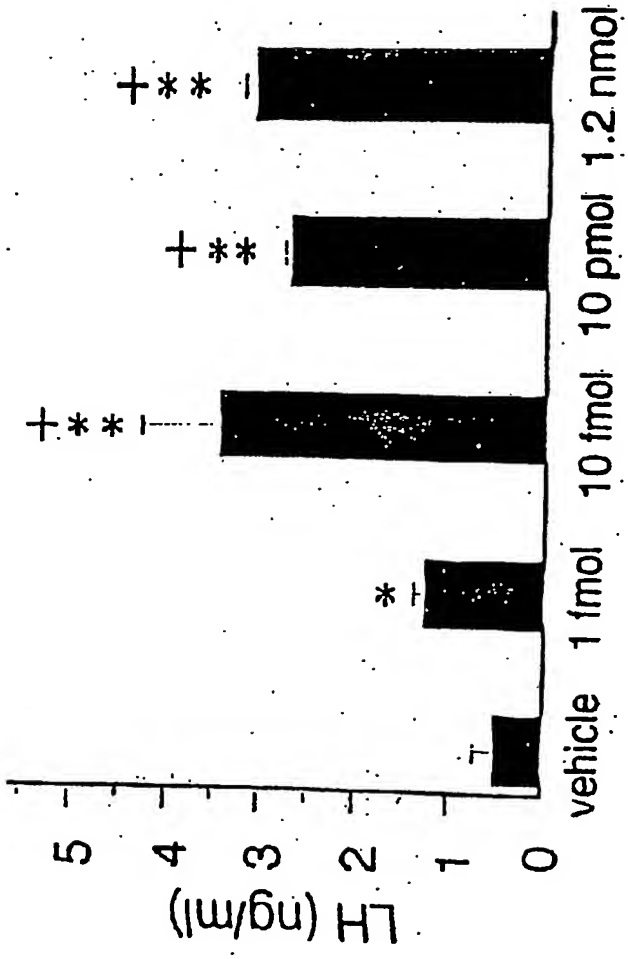

LH (ng/ml)
0
1
2
3
4
5
vehicle
1 fmol
10 fmol
10 pmol
1.2 nmol
Dose of Kisspeptin-54

FIGURE 3

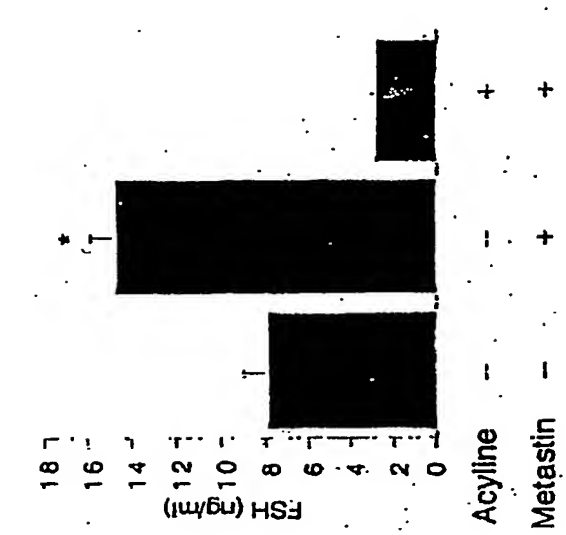


FIGURE 4B

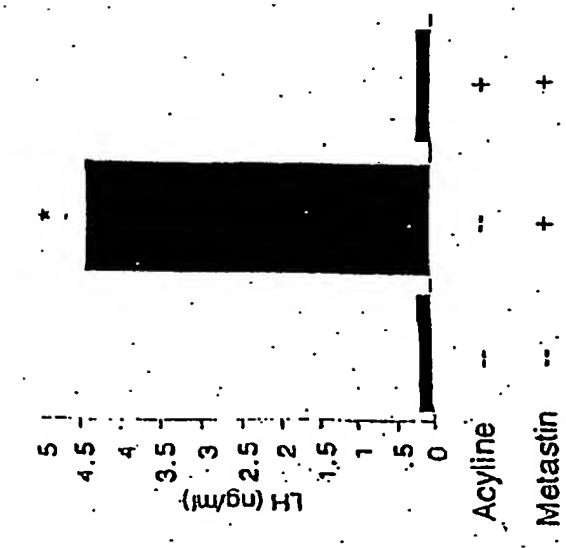


FIGURE 4A

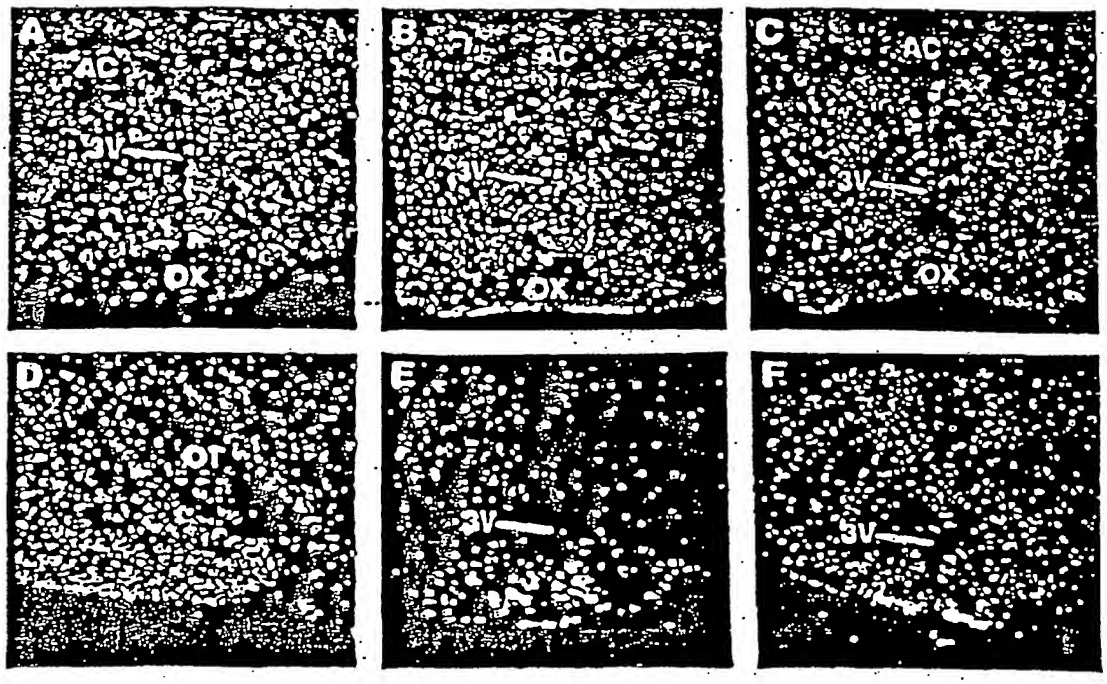


FIGURE 5

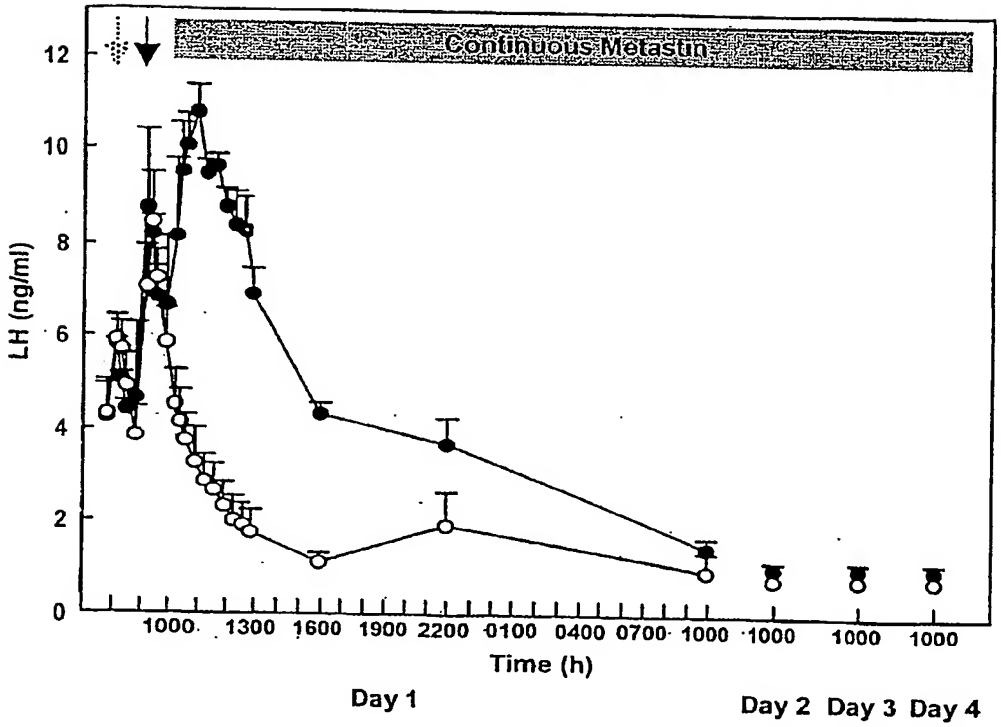

Continuous Metastin
LH (ng/ml)
12
10
8
6
4
2
0
1000 1300 1600 1900 2200 0100 0400 0700 1000 1000 1000 1000
Time (h)
Day 1
Day 2 Day 3 Day 4

FIGURE 6

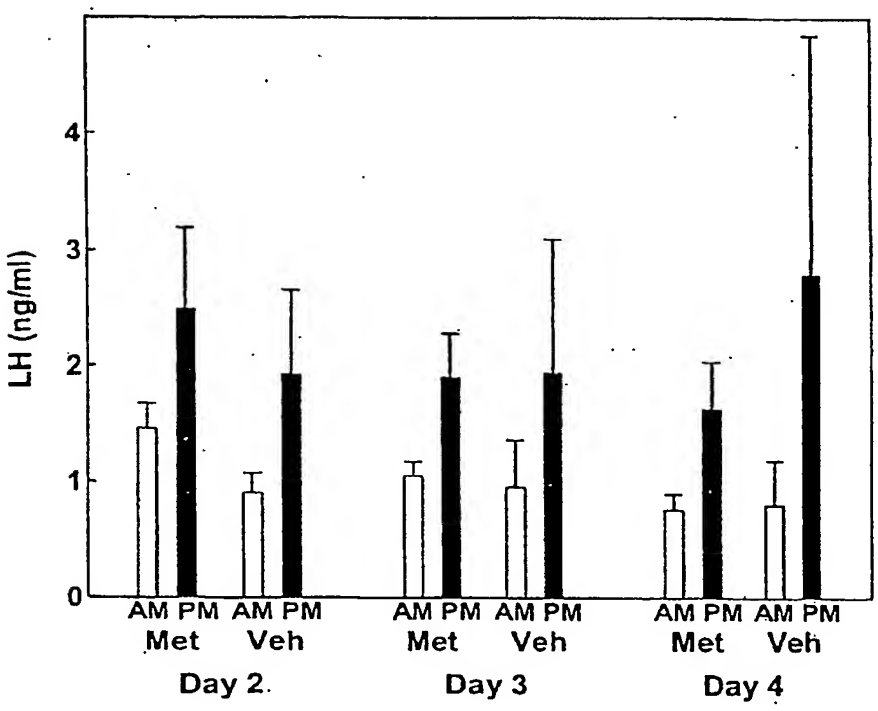

LH (ng/ml)
0
1
2
3
4
AM PM AM PM
Met Veh
Day 2
AM PM AM PM
Met Veh
Day 3
AM PM AM PM
Met Veh
Day 4

FIGURE 7

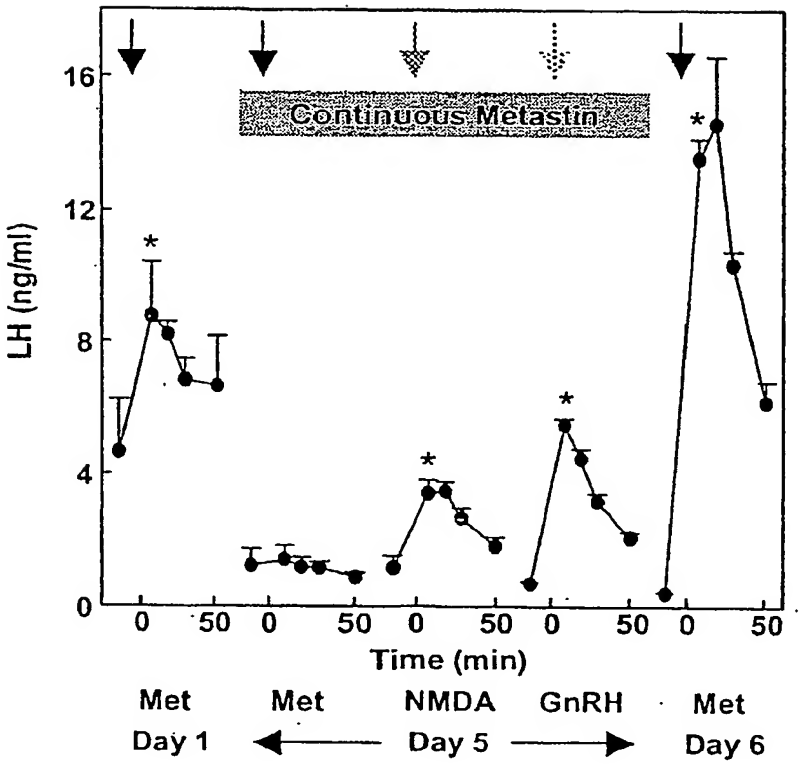

Continuous Metastin
LH (ng/ml)
16
12
8
4
0
0 50 0 50 0 50 0 50 0 50
Time (min)
Met
Met
NMDA
GnRH
Met
Day 1
Day 5
Day 6

FIGURE 8

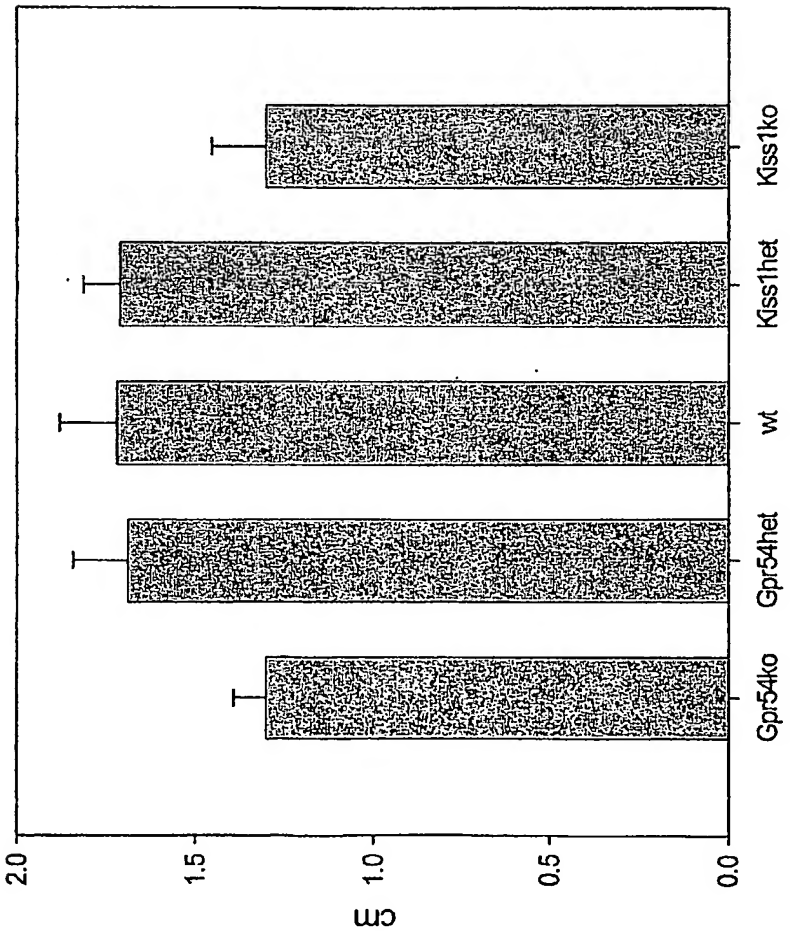


FIGURE 9

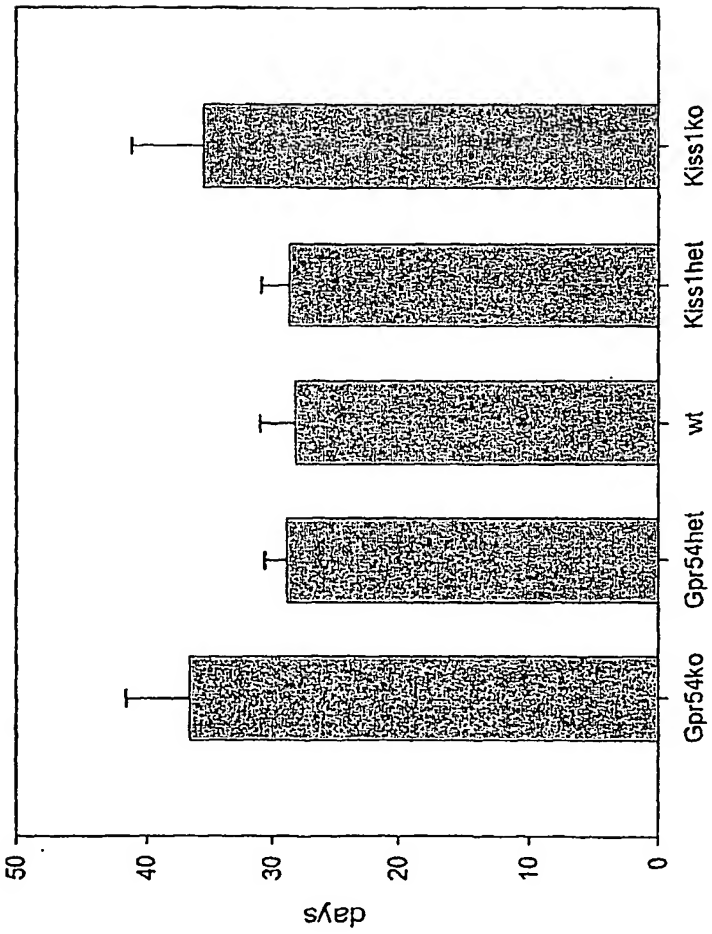
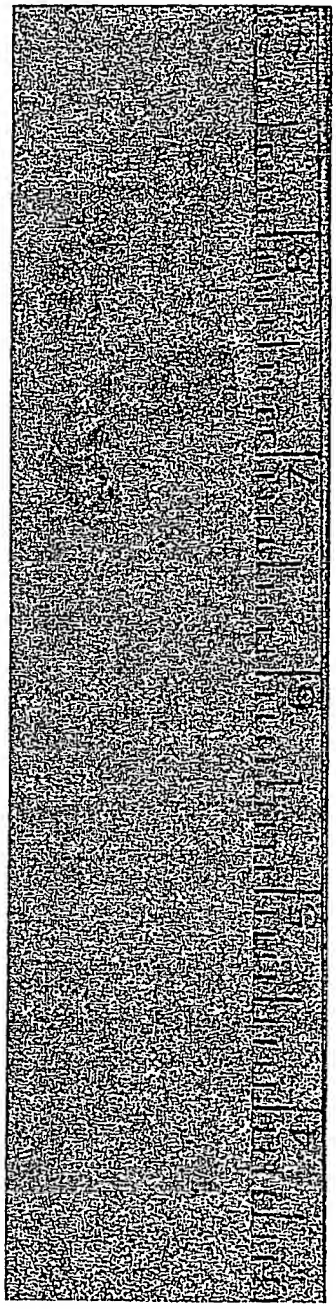
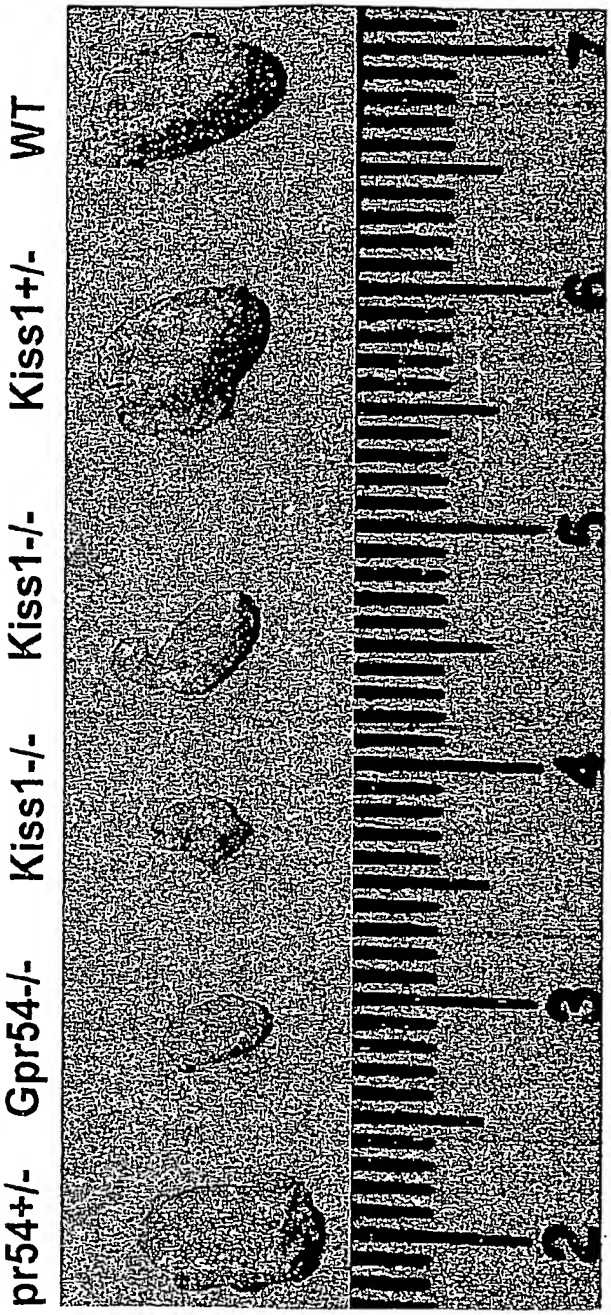


FIGURE 10

A



B



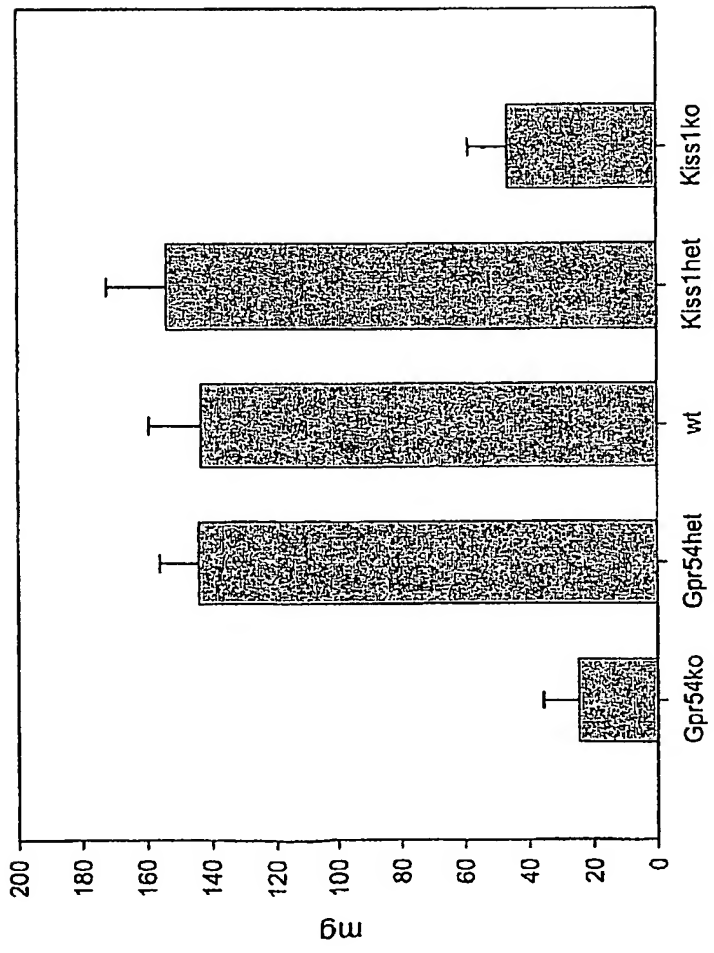

200
180
160
140
120
100
80
60
40
20
0
mg
Gpr54ko
Gpr54het
wt
Kiss1het
Kiss1ko

FIGURE 12

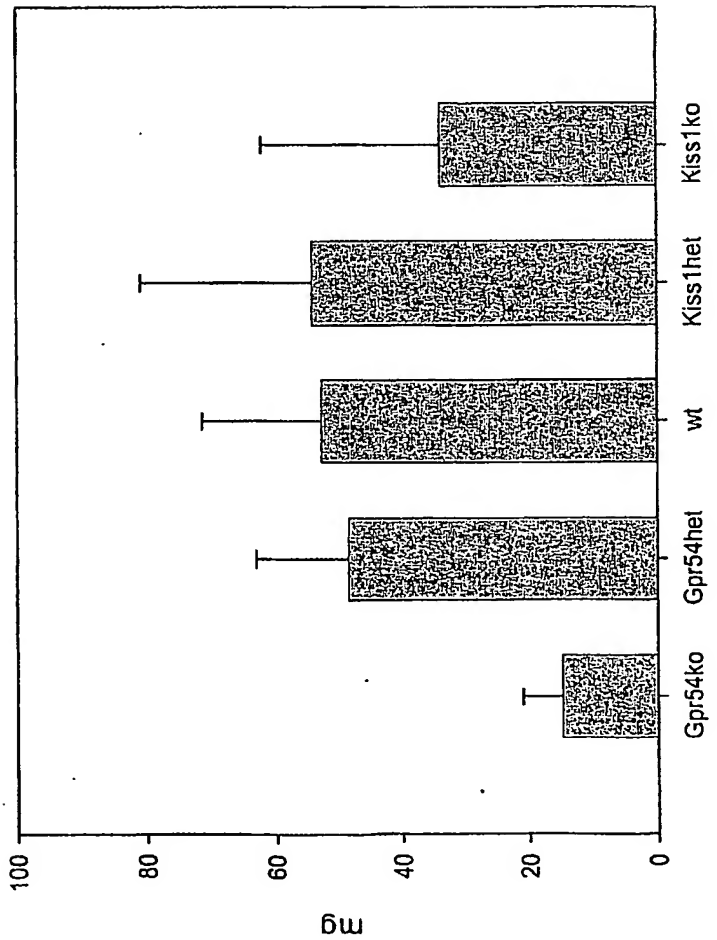


FIGURE 13

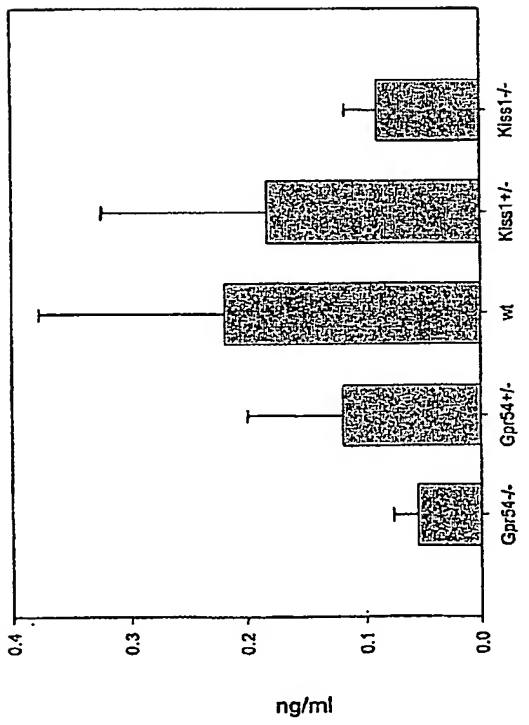

A
ng/ml
0.4
0.3
0.2
0.1
0.0
Gpr54-/-
Gpr54+/-
wt
Kiss1+/-
Kiss1-/-
B
ng/ml
35
30
25
20
15
10
5
0
Gpr54-/-
Gpr54+/-
wt
Kiss1+/-
Kiss1-/-

FIGURE 14

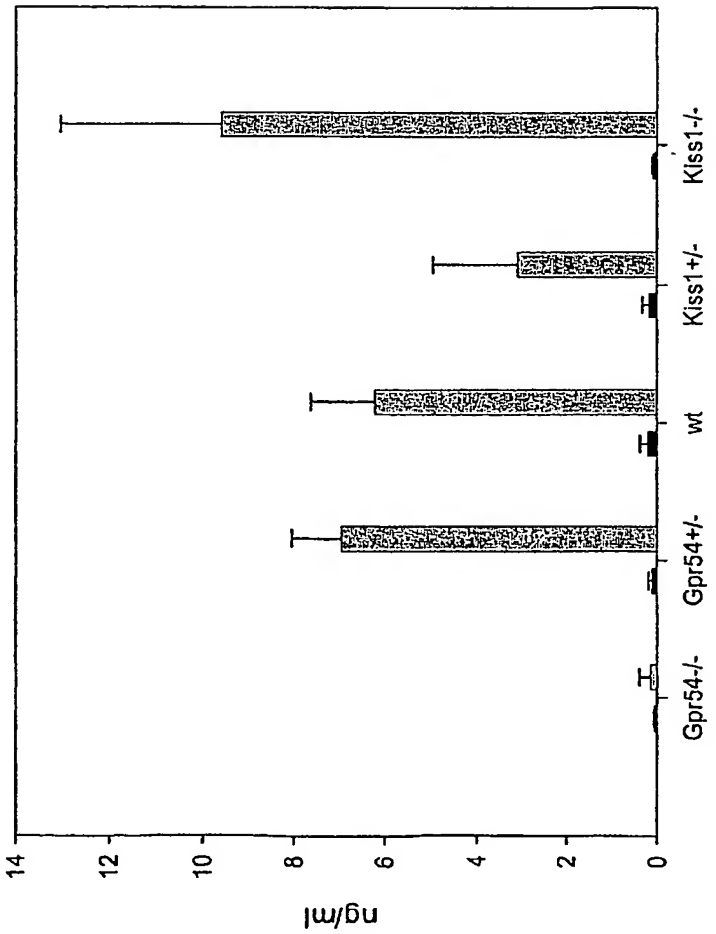


FIGURE 15

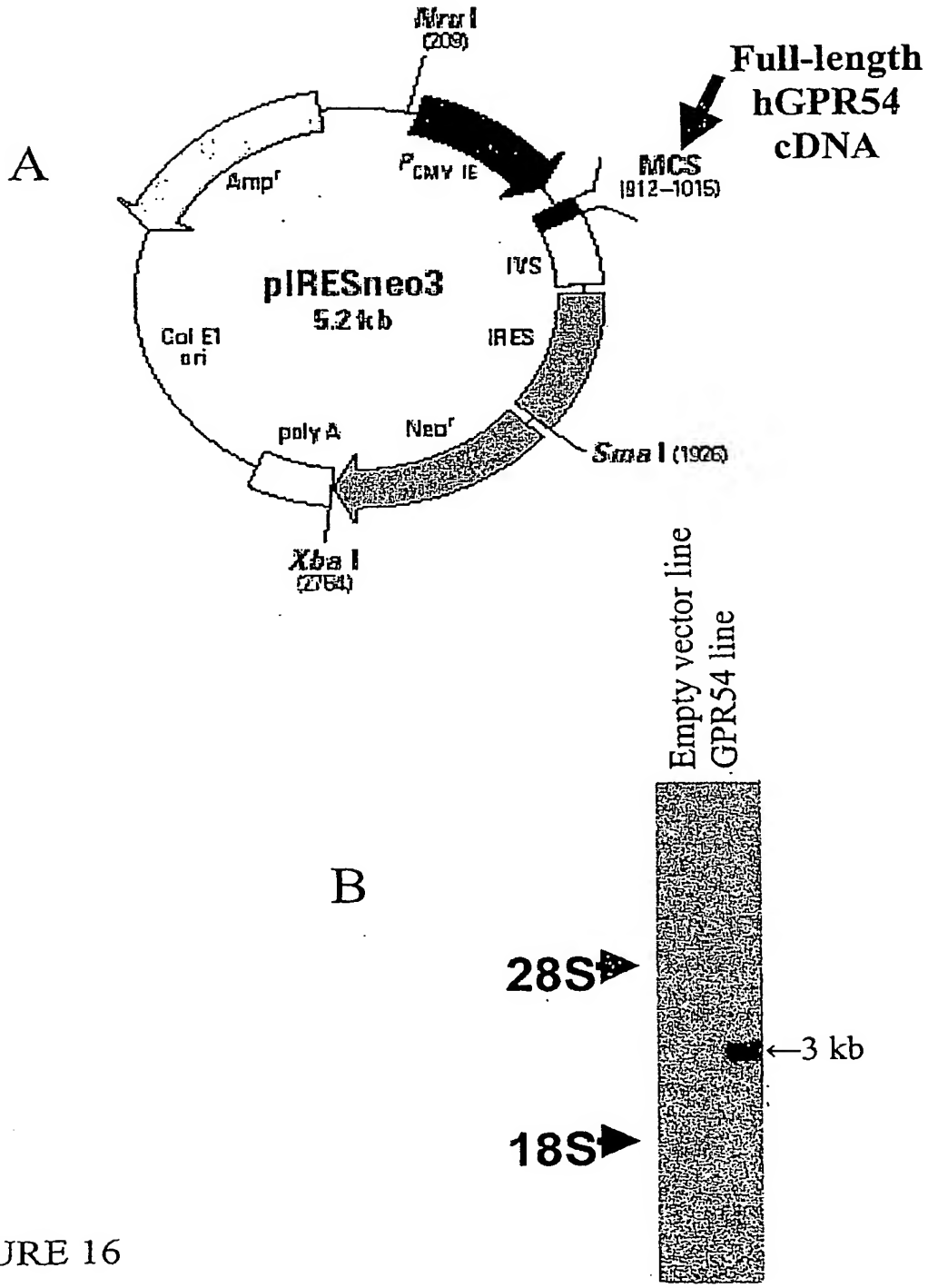

A
Mrn I
(209)
Full-length
hGPR54
cDNA
MCS
(912–1015)
Amp^r
P CMV IE
IVS
pIRESneo3
5.2 kb
Col E1
ori
IRES
poly A
Neo^r
Sma I (1926)
Xba I
(2754)
B
Empty vector line
GPR54 line
28S
18S
←3 kb

FIGURE 16

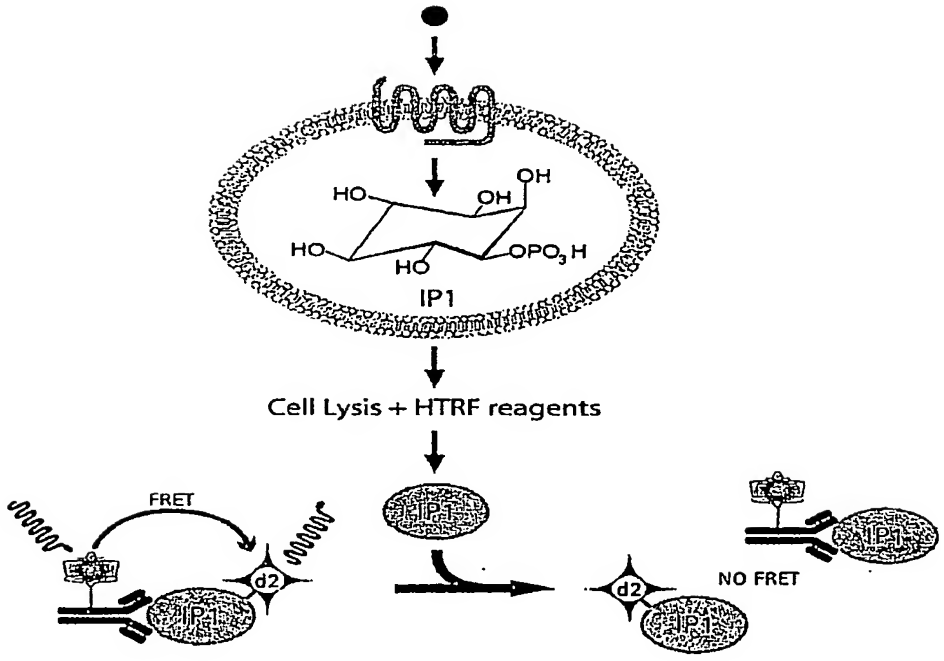

HO
OH
OH
HO
HO
OPO3H
IP1
Cell Lysis + HTRF reagents
FRET
d2
IP1
IP1
d2
IP1
NO FRET
IP1

FIGURE 17

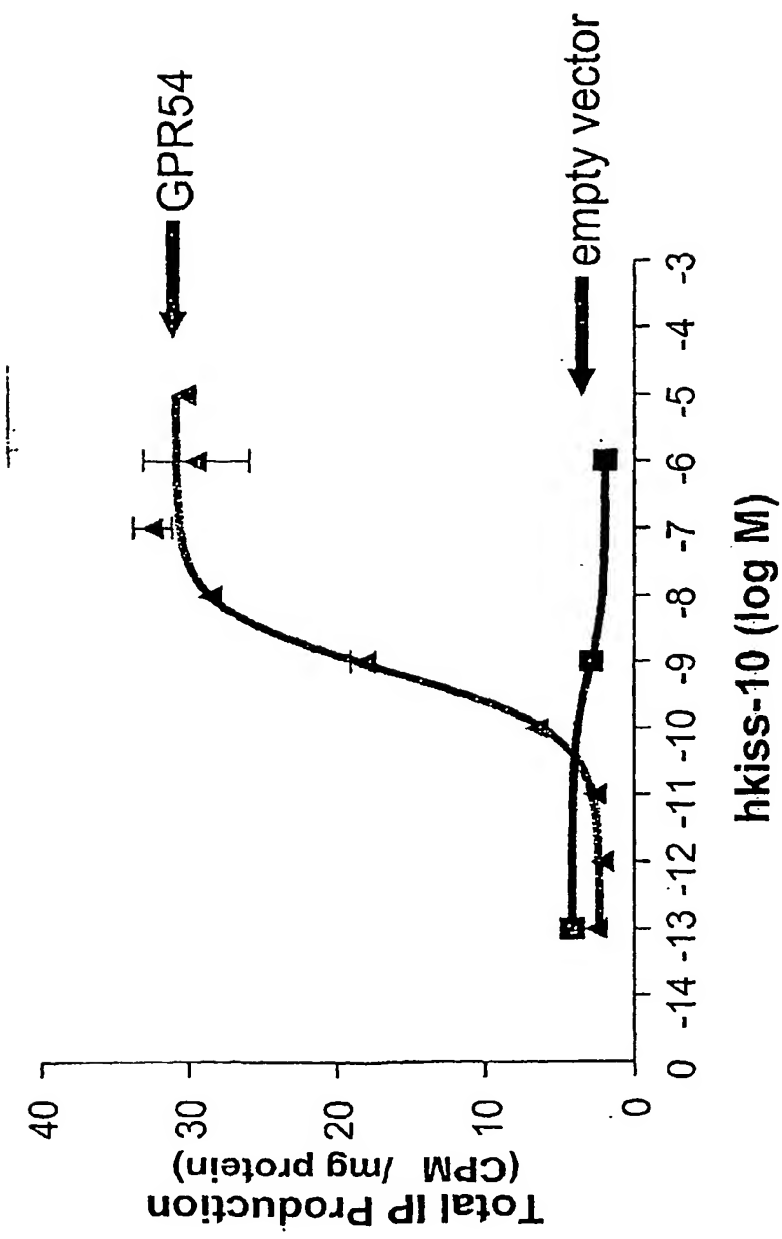

GPR54
empty vector
Total IP Production
(CPM /mg protein)
40
30
20
10
0
0 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4 -3
hkiss-10 (log M)

FIGURE 18

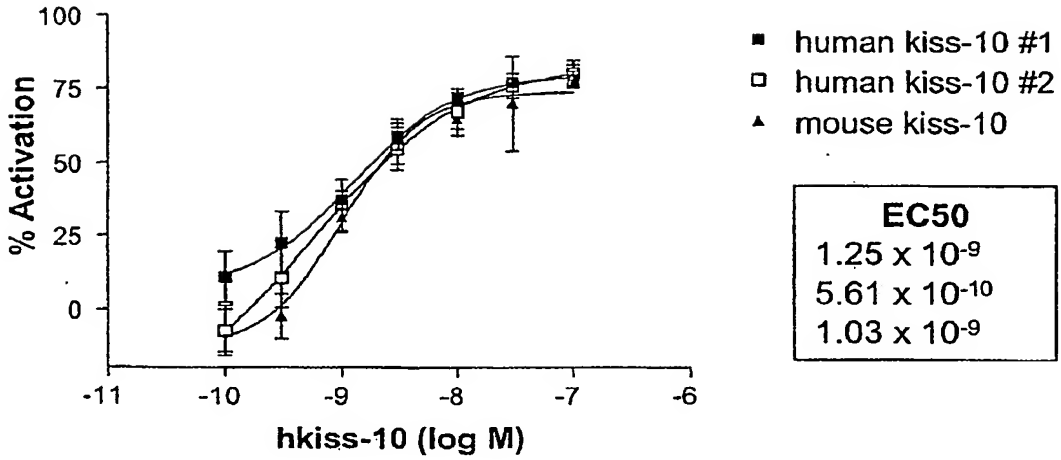
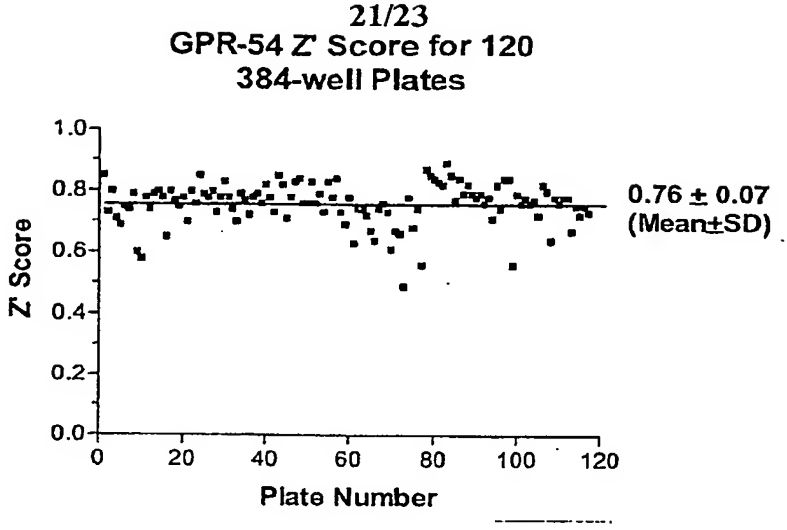
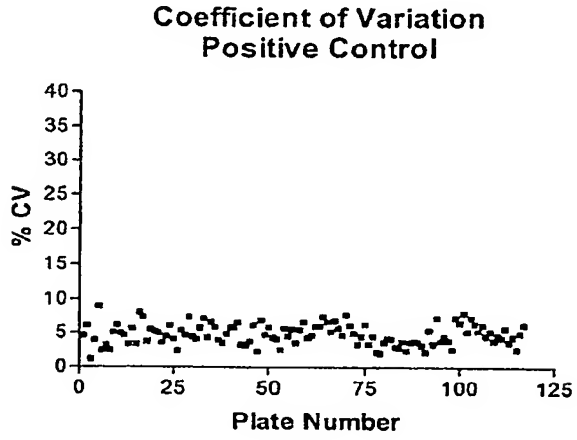

100
75
50
25
0
% Activation
-11
-10
-9
-8
-7
-6
hkiss-10 (log M)
human kiss-10 #1
human kiss-10 #2
mouse kiss-10
EC50
1.25 x 10-9
5.61 x 10-10
1.03 x 10-9

FIGURE 19

A



B



C

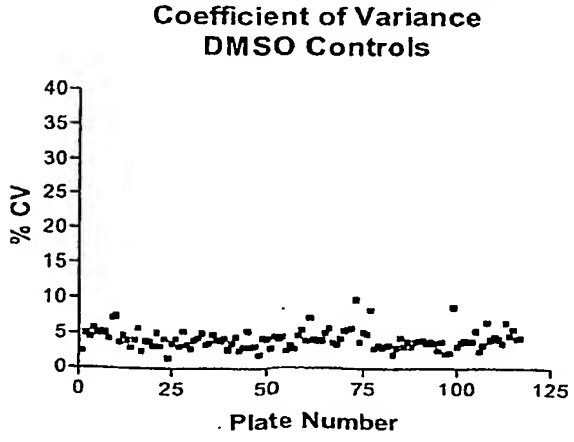
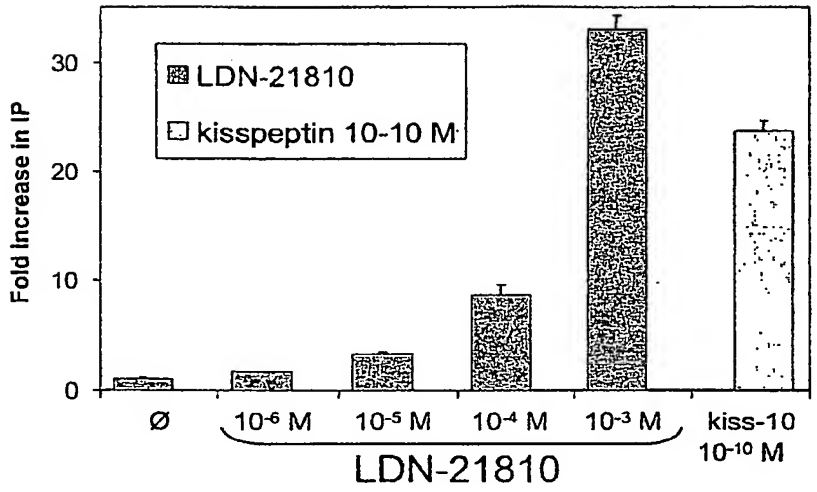


FIGURE 20

A


LDN-21810
kisspeptin 10-10 M
Fold increase in IP
30
20
10
0
Ø
10-6 M
10-5 M
10-4 M
10-3 M
kiss-10
10-10 M
LDN-21810

B

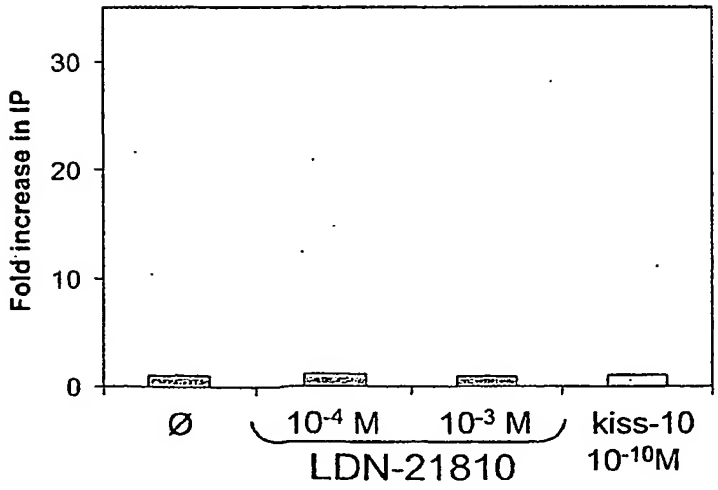

Fold increase in IP
30
20
10
0
Ø
10-4 M
10-3 M
kiss-10
10-10M
LDN-21810

FIGURE 21

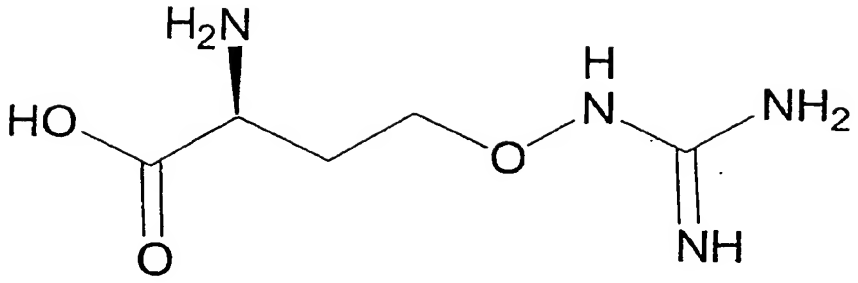


FIGURE 22

SEQUENCE LISTING

<110> The General Hospital Corporation
The Brigham and Women's Hospital, Inc.
University of Pittsburgh of the Commonwealth System of Higher Education et al.

<120> Use of GPR54 Ligands for Treatment of Reproductive Disorders, Proliferative Disorders, and for Contraception

<130> 00786/481WO2

<150> US 60/757,812
<151> 2006-01-09

<150> US 60/735,693
<151> 2005-11-11

<160> 2

<170> PatentIn version 3.3

<210> 1
<211> 145
<212> PRT
<213> Homo sapiens

<400> 1

Met Asn Ser Leu Val Ser Trp Gln Leu Leu Leu Phe Leu Cys Ala Thr
1 5 10 15

His Phe Gly Glu Pro Leu Glu Lys Val Ala Ser Val Gly Asn Ser Arg
20 25 30

Pro Thr Gly Gln Gln Leu Glu Ser Leu Gly Leu Leu Ala Pro Gly Glu
35 40 45

Gln Ser Leu Pro Cys Thr Glu Arg Lys Pro Ala Ala Thr Ala Arg Leu
50 55 60

Ser Arg Arg Gly Thr Ser Leu Ser Pro Pro Pro Glu Ser Ser Gly Ser
65 70 75 80

Arg Gln Gln Pro Gly Leu Ser Ala Pro His Ser Arg Gln Ile Pro Ala
85 90 95

Pro Gln Gly Ala Val Leu Val Gln Arg Glu Lys Asp Leu Pro Asn Tyr
100 105 110

Asn Trp Asn Ser Phe Gly Leu Arg Phe Gly Lys Arg Glu Ala Ala Pro
115 120 125

Gly Asn His Gly Arg Ser Ala Gly Arg Gly Trp Gly Ala Gly Ala Gly
130 135 140

Gln
145

<210> 2
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<213> Homo sapiens

<400> 2

Tyr Asn Trp Asn Ser Phe Gly Leu Arg Tyr
1 5 10